GERM CELL STUDIES IN THE MALE FOX (VULPES FULVA)[1]

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FOUR PLATES (TWENTY-SEVEN FIGURES)

INTRODUCTION

Although spermatogenesis has been described for many mammals there is a conspicuous lack of recorded observations for those animals whose pelts are of considerable commercial value. The present investigation was undertaken to remedy this situation in part, made possible by a limited series of well fixed material, collected during the breeding period.

Wodsedalek ('31) in a short abstract, stated that the chromosome number for the fox is 42 (diploid) and that the entire process of spermatogenesis had been studied. The present material does not support this claim with respect to number, and a more complete description has not been published. For these reasons it seems well to record the following observations.

MATERIALS AND METHODS

Testicular material from seventeen 1- to 5-year-old silver foxes, Vulpes fulva, was obtained by biopsy and autopsy at the U. S. Fur Animal Station, Saratoga Springs, New York, before, during, and after the mating period. Pieces of gonads were fixed in Bouin's fluid. Sections were cut at 5 and 8 microns. All material was stained in Heidenhain's or Delafield's haematoxylin.

[1] Wipf, L., and Shackelford, R. M., '42. Chromosomes of the Red Fox. Proc. Nat. Acad. Sci., vol. 28, pp. 265–268 appeared after Dr. Bishop's paper had been submitted and he had enlisted in the Army. Galley proof of this paper was read by Robert K. Enders and O. P. Pearson.

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Our experience has been that with this fixative, at least, good results were obtained only if small portions of the testis were immersed in the fluid. Whole testes, even with incisions through the tunica, were rarely satisfactory for cytological study. If a few seminiferous tubules, removed by biopsy, were immersed in Bouin's fluid, the fixation was very good.

The writer is indebted to Dr. Robert K. Enders, Swarthmore College, and to Mr. Charles Bassett, U. S. Fur Animal Station, for advice and cooperation during the course of this investigation. Thanks are due Mr. Oliver Pearson and Eleanor M. Paxson for much help in the preparation of material and figures.

ACTIVITY THROUGHOUT THE BREEDING SEASON

The breeding activity of the male fox, under the conditions in which these are raised, lasts from the end of January to late in March. Spermatozoa, presumably mature, were found in material taken late in November and in the middle of April, that is, considerably before and after the period of mating.

Gonads fixed in late November and early December showed a few dividing spermatogonia and spermatocytes, and some sperm. Most of the tubules, however, were inactive and contained no sperm. Tubules removed the last week in February upon investigation showed much activity among both spermatogonia and spermatocytes (figs. 1, 3, 5, 7, 14). Spermatozoa were found in all stages of transformation at this time. Material taken in late March still had a good many dividing spermatocytes and mature spermatozoa. By this time there was little activity among the spermatogonia and only rarely did they show division. Gonads removed in the middle of April contained some tubules in which the spermatocytes were still undergoing division and apparently normal sperm were being formed. However most of the tubules had ceased activity (fig. 2). Summarizing, it may be stated that spermatogenesis and sperm formation are not limited to the breeding

period, but are in progress at least 2 months before and 1 month after the mating season, although at a much reduced rate compared to that prevalent in the middle of the period.

ARRANGEMENT OF THE GERM CELLS WITHIN THE TUBULE

The arrangement of the cells within the tubule is very much like that described for the dog by Malone ('18). In the active testis groups of dividing cells are scattered at random along the length of the tubule. These patches of mitotic cells rarely surround the tubule completely. The number of dividing spermatogonia in an active region of a mature tubule varies between 80 and 256.

Dividing spermatogonia and spermatocytes are rarely present in the same region of a tubule, although first and second meiotic divisions are frequently found together. The spermatogonia are usually at the periphery of the tubule (figs. 5, 7), although occasionally, in rapidly dividing tissue, they may be located well within the tubule (fig. 1). The spermatocytes, on the other hand, frequently are found at the periphery as well as toward the lumen of the tubule, depending on the maturity of the testis (figs. 3, 14).

SPERMATOGONIA

The interkinetic germ cells and the Sertoli cells may be distinguished by their cytoplasmic characteristics (figs. 6, 14). The nuclei, however, are quite similar. A large nucleolus is usually present. In the spermatogonia this may be subdivided into as many as five separate nucleoli, which during prophase appear to be incorporated into the chromosome threads.

The germ cells of the fox are small and the chromosomes are crowded during division, so that counting is difficult. However in early metaphase stages of the spermatogonia the complete complex can be resolved. Thirty-two chromosomes are present forming a closely graded series (figs. 15, 16, 17). All of the larger ones are approximately V-shaped (atelomitic).

The spermatogonia in an active region are usually in the same stage of mitosis, suggesting an innate rhythm or intercellular coordination. Metaphases and anaphases are characterized by distinct spindles, but lack centrioles and centrosomes.

A. Chromosome pairing

The association of homologous chromosomes in diploid cells — "somatic pairing" — has been described for a number of plants and animals, particularly in the Diptera by Metz ('16) and Ribbands ('41). A polar view of a fox spermatogonium at metaphase shows what appears to be somatic pairing, or the juxtaposition of homologous members, in about ten pairs of chromosomes (fig. 15). This interpretation is not easy to verify owing to the scarcity of cells in a similar stage of development and the slight variations in size and shape of the chromosomes. However the larger chromosome pairs frequently show this association, considerably more often than would be expected on a basis of chance.

The significance of this phenomenon, should it prove to be of general occurrence, lies in the evidence brought to bear on questions of chromosome behavior and cell mechanics. One interpretation that naturally suggests itself is that pre-synaptic attraction of the homologs is expressed in the spermatogonia at this time.

SPERMATOCYTES

A. The growth period

The nuclear changes during the prophase of the first meiotic division have been followed from a pre-synaptic stage through diakinesis. In general the process closely corresponds to the description given by Painter ('24) for the opossum, to which the reader is referred.

The first recognizable stage may be regarded as the leptotene (fig. 9), in which the long intertwined chromosome threads are unpaired. At least one nucleolus is present, which may

be traced through the growth period, and is believed to give rise to the X-Y tetrad chromosome. Pairing of the leptotene threads was rarely observed and then only for two or three chromosomes in the nucleus. The early pachytene chromosomes are very fuzzy and apparently do not fix well (fig. 10). A considerable increase in the size of the nucleus takes place during the leptotene and pachytene stages. The karyotheca is very indistinct from the pachytene stage on; it is not clear just when the membrane disappears.

The double chromosome threads may be traced through a "bouquet stage" (fig. 11) and diplotene stages (figs. 12 a–d) during which the chromosomes gradually shorten and thicken. With this condensation of the threads the chromosomes lose their orientation, characteristic of the "bouquet stage."

At diakinesis, a brief period considering the relatively few cells found in this stage, the chromosomes are usually poorly stained and indistinct, and rarely show the four component chromatids (fig. 13). A rapid condensation of the chromosomes precedes their arrangement on the spindle at metaphase.

B. First meiotic division

The sixteen tetrad chromosomes at early metaphase are quite compact and spaced so that counts can be made (figs. 18, 19, 20). No particular features distinguish the individual chromosomes at this time.

At early anaphase the chromosomes can be counted without difficulty when viewed in lateral aspect (fig. 22). Certain tetrads are distinguished on a basis of size. Apparently all of the larger ones, at least, are atelomitic. One heteromorphic pair, probably the X-Y complex, is readily recognized.

A comparison of male and female haploid nuclei was afforded by the fortunate discovery of a fox oocyte fixed during the first meiotic division.[1] In figure 23 an oblique view of the spindle is shown, with the sixteen undivided tetrad chromosomes on the metaphase plate.

[1] The reproductive cycle of the female fox is being investigated by Doctor Enders and Mr. Pearson, to whom the author is indebted for the use of this material.

The sex-chromosome complex of the male consists of two unequal diads; the smaller is presumed to be the Y-chromosome, the larger, the X (figs. 4, 24). The latter is frequently bilobed, so that the entire tetrad is tripartite, a condition described in the opossum by Painter ('24). The segregation of the X and the Y diads is precocious (figs. 25, 26, 27). Ordinarily the segregation of the autosomal diads proceeds at such a rate that all the chromosomes reach the poles together.

C. Second meiotic division

After a brief interkinesis, during which the diads remain condensed, the chromosomes of the second spermatocyte rapidly divide to give rise to the nuclei of the spermatids. Relatively few cells were fixed while undergoing the second division. Figure 21 shows a second spermatocyte at metaphase with sixteen diad chromosomes. The X and the Y chromosomes can not be distinguished with certainty from the autosomes.

D. The chromatoid body

A chromatoid body is present in the cytoplasm of the spermatocyte during the leptotene stage (fig. 9). It may be traced through both meiotic divisions and into one-fourth of the spermatids (figs. 3, 6, 10, 21, 25), all the while apparently decreasing in size. During the transformation of the spermatid the chromatoid body is eliminated with the excess cytoplasm cast off from the tail of the sperm (fig. 6).

The behavior of the chromatoid body in the fox is similar to that described in the horse by Wodsedalek ('14) and in certain insects by Wilson ('13), Plough ('17), and Long ('40).

DISCUSSION

My observations confirm those of Wodsedalek in regard to the configuration and the behavior of the sex chromosomes. A small Y and a larger bipartite X segregate during the first meiotic division. The discrepancy in our chromosome counts lacks explanation. Frequently chromosomes stick together

and two might thereby be considered as one, but such cells, when detected, have been passed over in counting. In several cases spermatogonial counts of 34 and 35 can be attributed to the initial segregation of two or three pairs of daughter chromosomes.

In several respects spermatogenesis in the fox differs markedly from that in the dog, as described by Malone ('18), considering the fairly close phylogenetic relationship of the two animals. The male fox carries an X-Y sex-chromosome complex, whereas the dog was regarded as having the X-O type. The diploid number of chromosomes in the dog was stated by that author to be twenty-one, eleven less than that in the fox.

With respect to the growth period the outstanding differences between the fox and the dog appear to lie in the presence in the fox of a very prominent "bouquet stage" and a definite diakinesis stage, both common in mammalian spermatogenesis.

The arrangement of the germ cells in the tubules and the process of spermiogenesis as described by Malone in the dog are essentially like the conditions which exist in the fox.

It has been demonstrated that spermatogenic activity of the fox considerably exceeds the breeding period, in point of time, as far as the production of mature sperm is concerned. Spermatogenesis, per se, is not a practical criterion of male reproductive activity.

On the other hand it is clear that the activity of the germ cells is greatly reduced before and after the mating season, and may well cease entirely during the summer months. The decrease or absence of sperm formation at certain seasons of the year in annual breeders reported for some animals and animal hybrids (Dice, '42) is not necessarily a sign of the inability to produce sperm and of sterility.

SUMMARY

Germ cell activity in the silver fox, Vulpes fulva, shown by the presence of dividing spermatogonia and spermatocytes and of mature sperm, continues throughout the breeding season

from January to March. Mature germ cells are formed, at a reduced rate, 2 months before and 1 month after matings.

The diploid number of chromosomes in the fox is 32. Somatic pairing of homologous chromosomes in the diploid spermatogonia is believed to occur. The paired chromosomes have been traced through the growth period. The sex-chromosome complex in the male consists of a small Y and a larger X which segregate during the first meiotic division. A chromatoid body, present in the cytoplasm during the growth period, is carried through both divisions and is ultimately discarded during spermiogenesis.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph of active tubule showing many spermatogonia at metaphase and anaphase; fixed late in February. × 200.

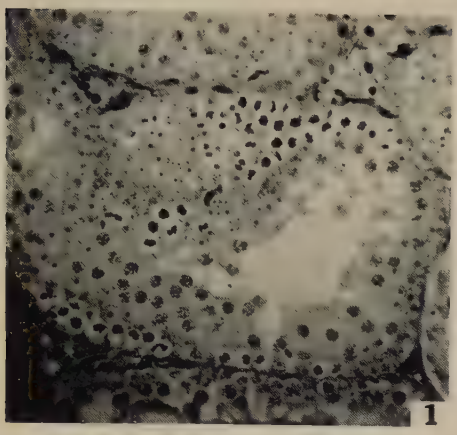
2 Inactive tubules after breeding season; no dividing cells or mature sperm. Some other tubules in this testis still active. × 180.

3 Longitudinal section of tubule with first (a) and second (b) spermatocytes, and developing sperm (d). Chromatoid body at (c). Fixed late in February. Compare figure 14. × 330.

4 First meiotic anaphase showing segregation of X and Y chromosomes. See figure 24. × 775.

5 Spermatogonia at periphery of tubule; same testis as figure 1. × 400.

6 Developing sperm showing acrosome (a) middle piece granule (b), chromatoid body (c), head, and tail piece. Spermatid (d) also shows chromatoid body. Cytosome of Sertoli cell at (s). × 775.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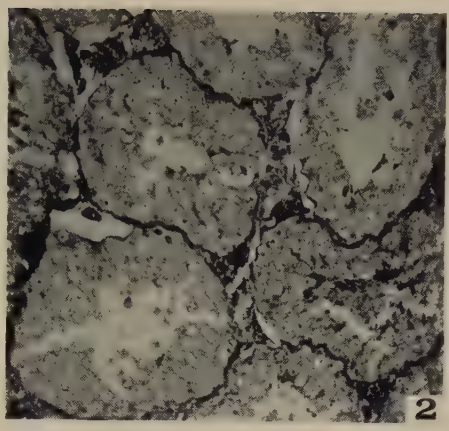
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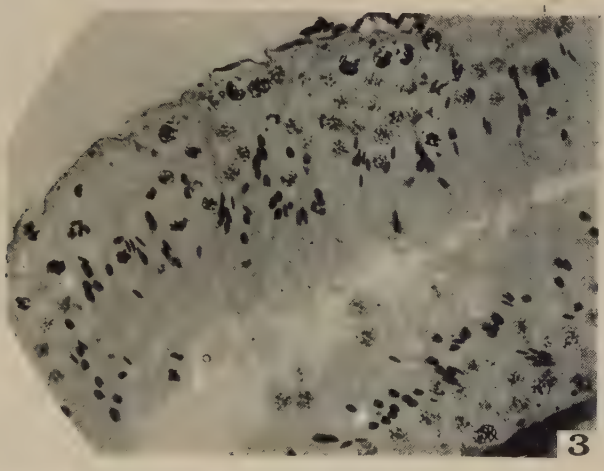
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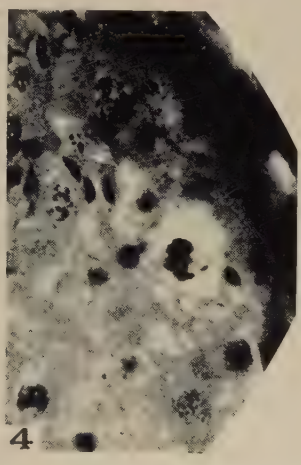
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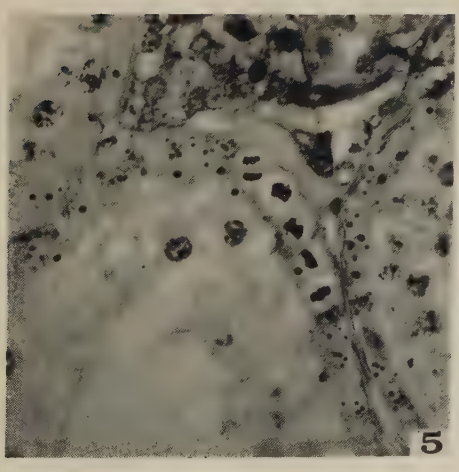
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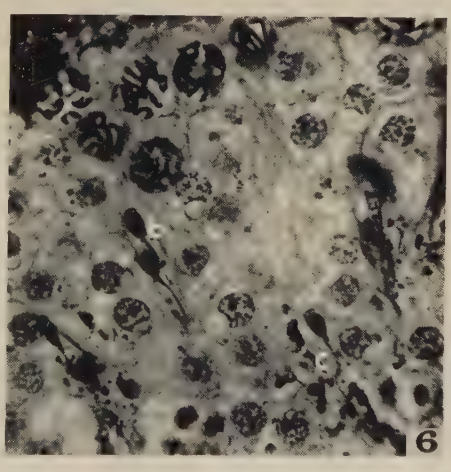
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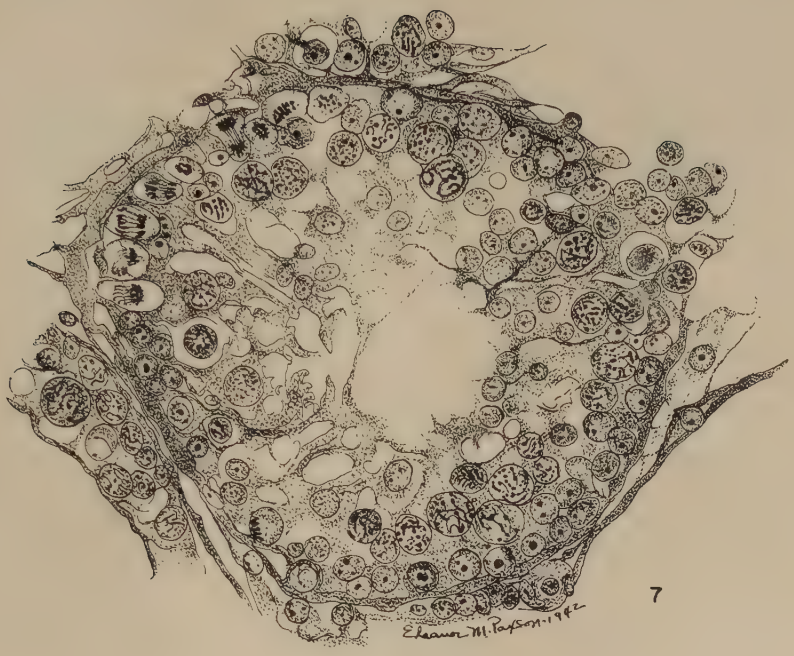
PLATE 2

EXPLANATION OF FIGURES

7 Tubule with spermatogonia dividing at periphery; growth stages toward lumen. Compare figure 5. × 330.

8 Mature tubule with sperm. Cytosome of Sertoli cell at (c). × 325.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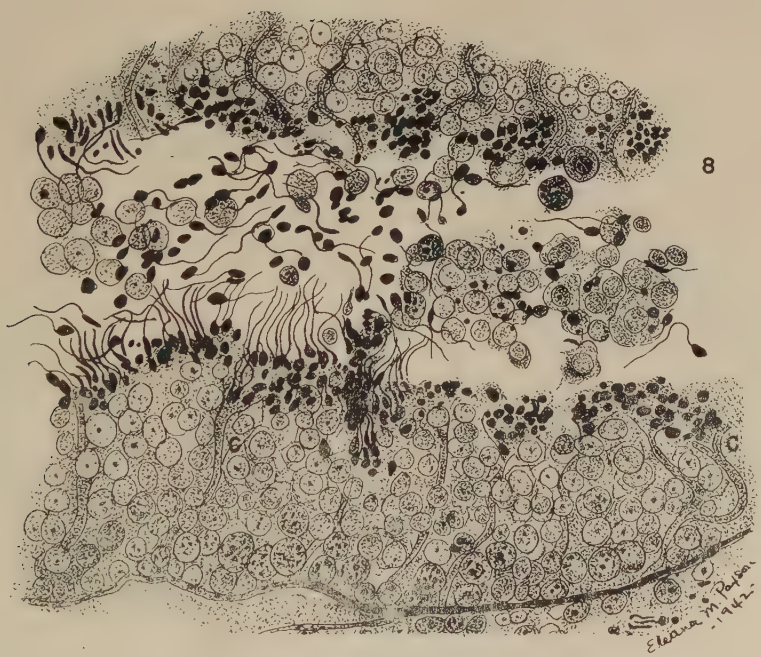
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PLATE 3

EXPLANATION OF FIGURES

9–13 reproduced about × 1735, 14 at × 330.

9 Leptotene stage with unpaired chromosome threads and nucleolus: chromatoid body at (c).

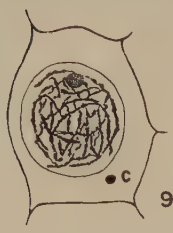
10 Paired pachytene chromosomes; chromatoid body (c).

11 "Bouquet stage" with looped chromosomes; nuclear membrane indistinct.

12 a–d Diplotene stages showing condensation of chromosome.

13 Diakinesis; six chromosomes drawn.

14 Tubule with first (a) and second (b) spermatocytes, spermatids, immature sperm, and Sertoli cells (s).



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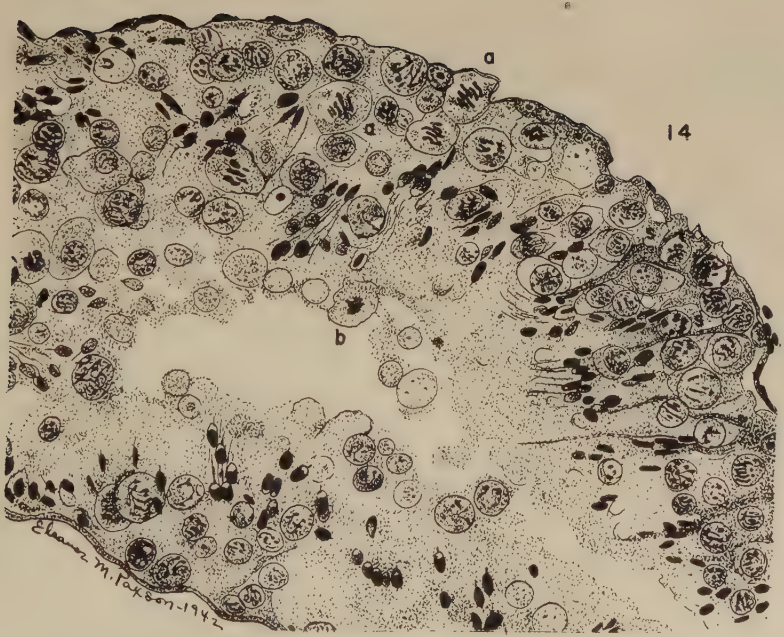

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PLATE 4

EXPLANATION OF FIGURES

All reproduced at about × 2,000

15 Metaphase plate of spermatogonium with thirty-two chromosomes. Arrangement suggests somatic pairing of homologs.
16 Spermatogonial metaphase drawn from two sections.
17 Same as 16.
18 Early metaphase of first spermatocyte with sixteen tetrad chromosomes and chromatoid body (c).
19 First spermatocyte metaphase.
20 First spermatocyte metaphase with chromatoid body (c).
21 Second spermatocyte metaphase showing sixteen chromosomes and chromatoid body (c).
22 Sixteen tetrads at early anaphase, lateral view; X–Y complex at extreme right.
23 First meiotic spindle of oocyte; sixteen tetrads at metaphase.
24 Segregation of X and Y chromosomes at first meiotic division. Same as figure 4.
25 Later stage in segregation of X and Y diads.
26 Same as 25. Chromatoid body at (c).
27 Same as 26.

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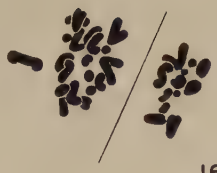

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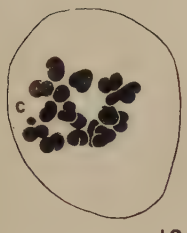

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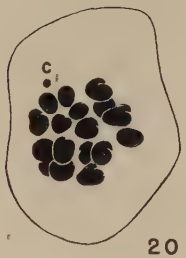

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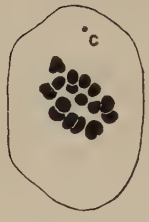

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THE LUNGS OF THE CETACEA, WITH SPECIAL REFERENCE TO THE HARBOR PORPOISE (PHOCAENA PHOCOENA, LINNAEUS)

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TWO TEXT FIGURES AND ONE PLATE (TWO FIGURES)

In two recent papers (Wislocki and Bélanger, '40; Bélanger, '40) comparisons were instituted between the lungs of several species of larger whales and those of certain dolphins and porpoises. It was pointed out that the lungs of Cetacea have certain features which characterize them all. Yet the lungs of the large whales differ in one important respect from those of the small Cetacea. It was demonstrated that the lungs of the large whales (Balaenoptera sibbaldii, Balaenoptera physalus, Physeter megalocephalus) possess excessively heavy myoelastic bundles in the septal borders of the air sacs and alveolar ducts, whereas no special system of myoelastic valves is present in the smaller bronchioles. In the smaller Cetacea (Delphinus delphis, Tursiops truncatus, Delphinapterus leucas), on the contrary, the smallest bronchioles have become differentiated into a highly specialized "valvular segment" (Bélanger, '40). This striking difference could have become the basis for a generalization, separating the whales from the dolphins and porpoises, were it not for the fact that Lacoste and Baudrimont ('33) have denied the presence of myoelastic sphincters in the terminal bronchiolar passages of the harbor porpoise (Phocaena communis). Because of this discrepancy the conclusion could not be drawn that uniformity exists among the dolphins and porpoises in regard to the presence of the "valvular segment."

MATERIAL AND METHODS

In consequence of this, the opportunity was welcomed recently to re-examine the lung tissue of a harbor porpoise. The material was placed at our disposal through the courtesy of Mr. Harry C. Raven of the American Museum of Natural History, who obtained the specimen (A. M. N. H. 2182 C. A) at Cape Breton Island and identified it as a harbor porpoise (Phocaena phocoena, Linnaeus). The lung tissue was examined by dissection under a binocular dissecting microscope, and sections were prepared, stained with hematoxylin and eosin, by the Azan technique, Masson's trichrome mixture, and by Weigert's resorcin fuchsin for elastic tissue. A drawing is submitted of the smallest bronchiolar segment of Phocaena, together with one, at identical magnification, of a specimen of the lungs of Delphinus delphis which possesses highly typical "valvular segments." The lungs of this common ocean dolphin (Delphinus delphis) were obtained through the courtesy of Mr. Arthur F. McBride of Marine Studios, Florida. The lung tissue of the latter was prepared for study in the same way as that of the harbor porpoise.

OBSERVATIONS

Figure 1 illustrates the morphology of the smallest bronchiolar passages of the harbor porpoise and should be compared with the drawing, at identical magnification, of the same segment of the dolphin's lung. It will be observed that the smallest bronchioles of the harbor porpoise do possess sphincter valves, although they are distinctly less numerous and more delicate than those of Delphinus (fig. 2), or Tursiops and Delphinapterus (Wislocki and Bélanger, '40; Bélanger, '40). In the harbor porpoise there does not appear to be a succession of more than eight to twelve valves in a single terminal bronchiolar passage, whereas in the larger porpoises and dolphins as many as several dozen are present in a valvular segment. Because of this relative restriction of the extent of the valvular segments in the harbor porpoise, valves are not encountered as frequently in ordinary histological sections as they are in

lung sections of the somewhat larger related species. Yet in sections, especially when stained by the Azan method, Masson's trichrome mixture, or with Weigert's resorcin fuchsin, instead of with hematoxylin and eosin, typical myoelastic

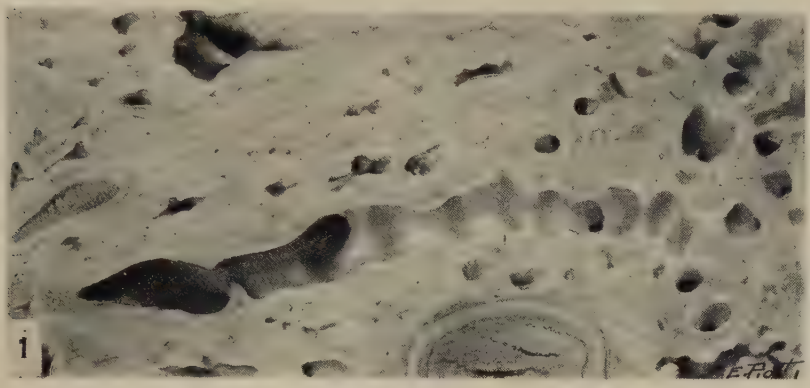

Fig. 1 A drawing of a valvular segment of the bronchiolar tree of a harbor porpoise, showing the characteristic myoelastic sphincters subdividing the bronchiole into compartments. × 14.

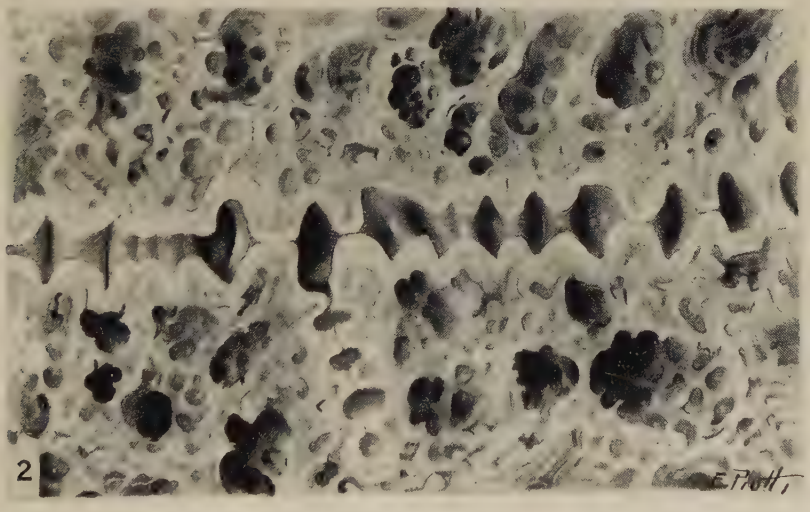

Fig. 2 A drawing of a valvular segment of the bronchiolar tree of a dolphin, at the same magnification as the preceding figure, to illustrate the identity of the valvular segment in these two species. Observe that the myoelastic sphincters are more numerous and larger than in the harbor porpoise. × 14.

valves can be recognized with no great difficulty (figs. 3 and 4). Thus, contrary to the statement of Lacoste and Baudrimont, the lungs of the harbor porpoise possess typical valvular segments similar to those of other porpoises and dolphins which have been examined.

In other respects, also, the structure of the lungs of the harbor porpoise is identical with that of Delphinus, Tursiops and Delphinapterus. As in the latter, the lungs are characterized by the presence of cartilaginous armature extending down to the respiratory sacs, by the occurrence of a double capillary net in the interalveolar septa, and by a relatively large amount of collagenous and elastic tissue forming the supporting framework of the bronchi, blood vessels and air sacs. Thus we reach the conclusion that the essential lung structure of the harbor porpoise is identical with that of other porpoises and dolphins which have been investigated.

We are now in a position for the first time to propose the generalization that the lungs of the porpoises and dolphins differ materially from those of whales by the evolution in the smallest bronchiolar passages of a specialized valvular segment containing myoelastic sphincters. The characteristic features and homologies of the valvular segment have been described and discussed at sufficient length in previous papers (Wislocki and Bélanger, '40; Bélanger, '40) to make it unnecessary to recapitulate them here.

SUMMARY

The essential lung histology of the harbor porpoise (Phocaena phocoena) is identical with that of other porpoises and dolphins which have been investigated. Like the latter, the lungs of the harbor porpoise are characterized by the possession of a specialized system of myoelastic sphincters constituting a "valvular segment," involving the smallest bronchiolar passages. The conclusion is reached that a specialized valvular segment is characteristic of the lungs of porpoises and dolphins, in contrast to whales, the lungs of which do not exhibit this specialized structure.

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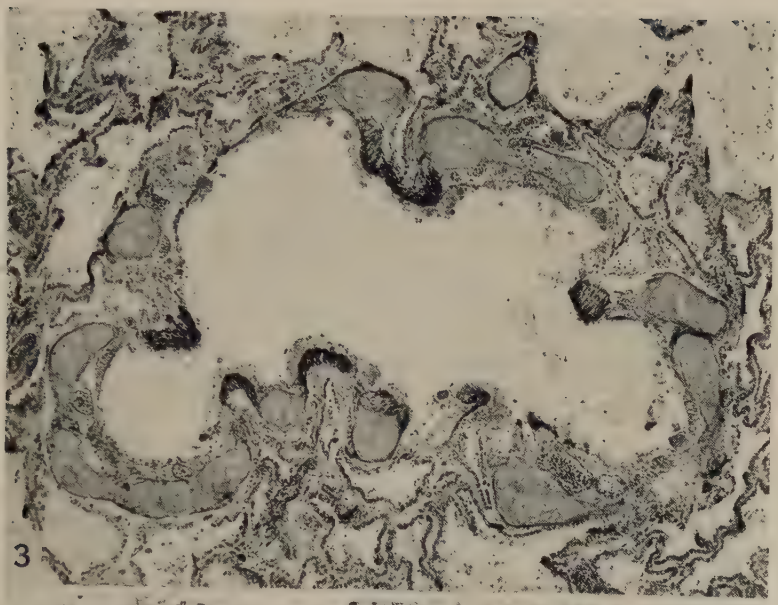
PLATE 1

EXPLANATION OF FIGURES

3 Section of a lung of a harbor porpoise stained for elastic tissue (Weigert's resorcin fuchsin), showing several myoelastic sphincters constricting a bronchiole. × 70.

4 Section of a lung of a harbor porpoise stained by Masson's trichrome mixture, showing a characteristic myoclastic valve. × 150.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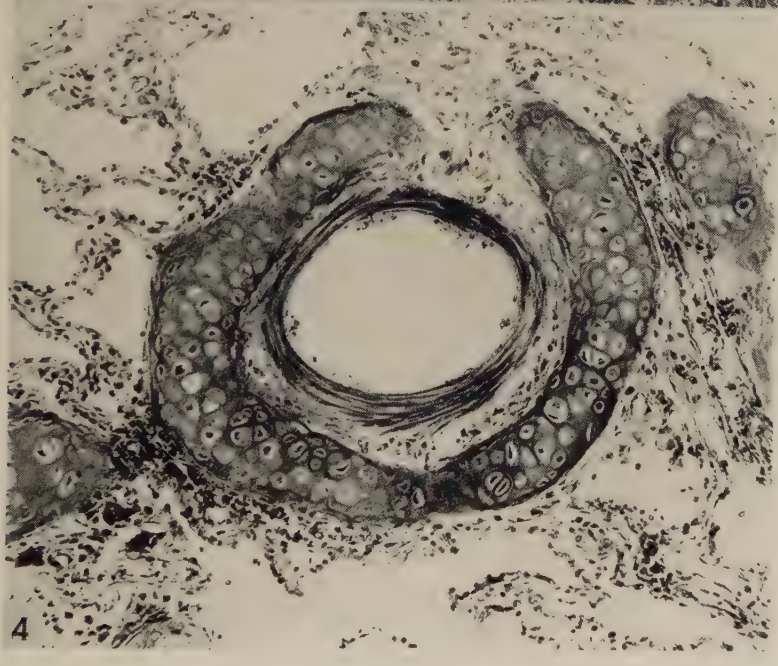
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INTRACELLULAR COLLOID IN THE INITIAL STAGES OF THYROID ACTIVATION

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TWO PLATES (NINE FIGURES)

Analyses of the early activation of the thyroid gland by the thyrotropic factor of the pituitary have been made chiefly on the thyroid of the guinea pig. No corresponding studies have been carried through on the rat, because it seemed to be rather unresponsive to the thyrotropic factor, particularly when studied with the common histological techniques. Yet abundant cytological changes do take place even in this animal, as was demonstrated by the use of the Altman-Gersh freezing-drying method followed by a simple staining technique (De Robertis, '41 a). With this technique the thyroid gland of the rat injected with thyrotropic factor shows a rapid change in the intracellular colloid. This follows a particular pattern and permits a deeper analysis of the cytology of this early activation to be made.

The injections of different doses, ranging between 0.5 to 10.0 guinea pig units, have been found to produce cytological changes that are very sensitive and so rapid as to make this method applicable with advantage in the assay of thyrotropic factor of the pituitary gland.

[1] The first part of this work was done while the author held a Fellowship of the Rockefeller Foundation. This research is part of a project supported by an allotment from the Rockefeller Fluid Research Fund.

METHODS

Twenty-two white rats were injected intraperitoneally with thyrotropic factor of the pituitary gland.[2] Doses and times are shown in table 1. Animals were killed by a blow on the head; the thyroid gland was removed immediately, immersed

TABLE 1

RAT NO.	DOSE IN THYROTROPIC G.P. UNITS	TIME AFTER INJECTION	NUMBER OF INTRACELL. COLLOID DROPLETS	NUMBER OF CYTOPLASMIC (SECRETORY?) EXTENSIONS TOWARD THE LUMEN
		hrs.		
1	0.5	½	+	+
2	1	½	+	+
3	3	½	++	++
4	5	½	+++	++
5	0.5	1	++	++
6	1	1	+++	+++
7	3	1	++++	+++
8	5	1	++++	++
9	10	1	++++	+++
10	0.5	3	—	—
11	1	3	—	—
12	3	3	+	—
13	5	3	+++	+
14	10	3	+++	+
15	10	3	+++	+
16	0.5	9	—	—
17	1	9	—	—
18	3	9	—	—
19	5	9	—	—
20	0.5	22	—	—
21	1	22	—	—
22	3	22	—	—
23	control		—	—
24	control		—	—

in liquid air and afterwards dried in a vacuum at —30°C. Glands were denatured in absolute alcohol and embedded in nitrocellulose. For the demonstration of intracellular colloid, sections were stained with aniline blue-orange G by the method previously described (De Robertis, '41 a).

[2] Thyrotropic factor was obtained from Doctor Jensen and contained 10 guinea pig units per milligram of dried powder.

OBSERVATIONS

Thirty minutes after the injection of thyrotropic factor. With 0.5 g. p. unit, intracellular colloid increases. It appears as large droplets in the apical part of the cells. Some of these droplets seem to pass into the follicular lumen.

With larger doses, activation is more evident. The form of the cells remains cuboidal, though a little higher than normal, and with a convex apical surface. Intracellular colloid increases. Some cells have smaller droplets, particularly near the nucleus in the apical zone. Indications of release of droplets into the lumen are very conspicuous. They approach the apical border, bulge out into the lumen, and seem to break into the colloid of the lumen by rupture of the cytoplasmic surface. More frequently a small part of the cytoplasm containing one or more droplets bulges into the lumen of the follicle. Rupture takes place by progressive thinning of the pedicle (figs. 2 and 5), until the colloid droplets remain in the lumen, surrounded for some time by a thin film of cytoplasm (fig. 5).

One hour after the injection of thyrotropic factor. With 0.5 or 1.0 unit, cells are cuboidal but higher than in normal glands, have convex surfaces at the apical pole and contain numerous colloid droplets. Simultaneously the number of cytoplasmic protrusions toward the lumen greatly increases (figs. 3, 6, 7).

With 3.0 g. p. units, the number of colloid droplets in the cytoplasm, and of cytoplasmic protrusions reaches a maximum which is not increased with higher doses of thyrotropic factor (5–10 units), (fig. 4).

Three hours after the injection of the thyrotropic factor. With 0.5 or 1.0 unit, activation is not very conspicuous. The cells are higher than normal, with few droplets in the apex. There are few cytoplasmic extensions into the lumen. Some cells show droplets of a pale staining colloid situated generally in the base of the cell. These resemble the Bensley cells with basal colloid in the opossum.

Following the injection of 3.0 g. p. units intracellular colloid is increased. The cells are columnar. Basal dilute colloid is more abundant. Apical bulges are very rare.

After the administration of 5.0 to 10.0 units, intracellular colloid increases enormously. It appears, generally, as large pale stained vacuoles. Some cells are so charged with colloid that they look like a sponge whose spaces are filled with dilute colloid (fig. 8).

Nine hours after the injection of thyrotropic factor. With 0.5 or 1 unit, the cells are cuboidal with convex apical surface. Intracellular colloid is not greater than in normal animals. When it appears, it is in the form of small droplets beside the nucleus and at the base. Few cells have basal dilute colloid. No apical irregularities are visible.

With 3 to 5 units, the cells are columnar, and contain few large colloid droplets. Very small droplets of darkly stained colloid are seen beside the nucleus and at the base, chiefly the latter. Few cells show a real morphological inversion of polarity with the nucleus in the apex and the base filled with colloid droplets.

Twenty-two hours after the injection of the thyrotropic factor. With 1 to 3 units, the cells are columnar. Intracellular colloid is distributed as small droplets near the nucleus and in the base. All intermediary stages of inversion of cell polarity are visible (fig. 9). At the same time intrafollicular colloid is diminished.

DISCUSSION

Based on the investigations carried out on guinea pigs, dogs, etc., it is generally accepted that the thyrotropic factor of the pituitary produces a rapid activation of the thyroid gland with hypertrophy of the cells, colloid reabsorption and release of thyroid hormone into the circulation (Thurston, '33; Wilcke, '35). As several authors showed that the rat organism is rather insensitive to thyrotropic hormone (Aron, '32; Houssay, Novelli and Sammartino, '32; Thurston, '33), cytological investigations on this animal were neglected. To

fill out this gap, we have tried to give an analysis of cytological processes in the rat thyroid.

Changes after the injection of thyrotropic factor have been recorded as early as 30 minutes (Krogh and Okkels, '33), 45 minutes (Ponse, '38; Starr and Metcoff, '41) and 2 hours (Eitel and Loeser, '32). In unpublished experiments, using the freezing-drying method, we found in the guinea pig thyroid a definite increase of intracellular colloid 15 minutes after the injection of the extract containing the thyrotropic factor.

There is a great lack in the literature of cytological data on the changes of intracellular colloid in this early activation. This is due to the fact that common histological methods tend to mask the colloid droplets in the cells (De Robertis, '41 a). For example Krogh and Okkels, who published the most complete cytological observations based on routine cytological material, described only a hypertrophy of the thyroid cells, definite changes in the Golgi apparatus and differences in the staining of the cytoplasm.

It is generally admitted that the thyrotropic factor stimulates the reabsorption of the stored colloid. Our results indicate that in early stages (with its maximum at 60 minutes) cells are actively secreting toward the lumen. Colloid droplets which are formed near the nucleus move toward the apex and, at the same time increase in size. It may be that the excretion of these droplets into the lumen is preceded by a diminution of the surface tension in some parts of the apical surface. At any rate, at these points, the cytoplasm enclosing the colloid droplets bulge into the lumen, to be released finally by rupture of the pedicle. These processes were observed also in the guinea pig. They suggest that thyroid cells produce an apocrine secretion, parts of their cytoplasm being excreted together with the secretion into the lumen.

After this phase the cells appear to stop secreting toward the lumen, seem to begin secretion toward the base, and reabsorb the stored colloid in the lumen. This assumption is further supported by the observations that (1) pale-staining, seemingly dilute colloid appears in the base of the cell, (2)

small colloid droplets are distributed in the basal half of the cell, resulting in a tendency toward a complete inversion of morphological polarity, and (3) intrafollicular colloid diminishes. Probably parallel to the inversion of polarity a physiological inversion also takes place (De Robertis, '41 c).

In a previous paper (De Robertis, '41 b), we showed that an enzymatic mechanism is probably involved in the reabsorption process of the colloid of the lumen. We found that in the colloid extracted from a single follicle is an enzyme whose proteolytic activity increases after injection of the thyrotropic factor. New experiments now under way suggest that during the first phase (secretion toward the lumen), the proteolytic activity of the colloid is not enhanced. This means that there exists a close correlation between the intensity of reabsorption and the increase of proteolytic activity of the colloid.

Assay of the thyrotropic factor

Direct estimations of thyrotropic factor of the pituitary have been based generally on weight or histological changes of the guinea pig thyroid. As the histological method is more sensitive and reliable (Heyl and Laqueur, '35), the manner of fixation is obviously of great importance (Van Dyke, '39).

The fact that the freezing-drying method followed by a simple staining technique (De Robertis, '41 a), permits the detection of the thyrotropic factor in doses as small as 0.5 guinea pig unit, or even less, leads us to suggest that this method could be applied with advantage to the assay of thyrotropic factor. A cytological method, based on the increase of intracellular colloid in this early activation would appear to be very sensitive and rapid.

SUMMARY

Changes in the intracellular colloid were studied in the thyroid glands of rats injected intraperitoneally with graded doses and at different time intervals with thyrotropic factor of the pituitary gland. In the first phase thyroid cells produce a great quantity of intracellular colloid which appears to be

secreted toward the follicular lumen. In a second phase colloid seems to be secreted toward the base and reabsorption of the intrafollicular colloid takes place. A parallelism between these processes and the variation in proteolytic activity of the colloid is described and an interpretation is suggested. The cytological method described is so sensitive and quick that it may be based with advantage in the assay of the thyrotropic factor.

I wish to acknowledge my indebtedness to Dr. I. Gersh for his advice and criticism, and to the Department of Anatomy of the Johns Hopkins Medical School for the facilities and courtesies I received.

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PLATE 1

EXPLANATION OF FIGURES

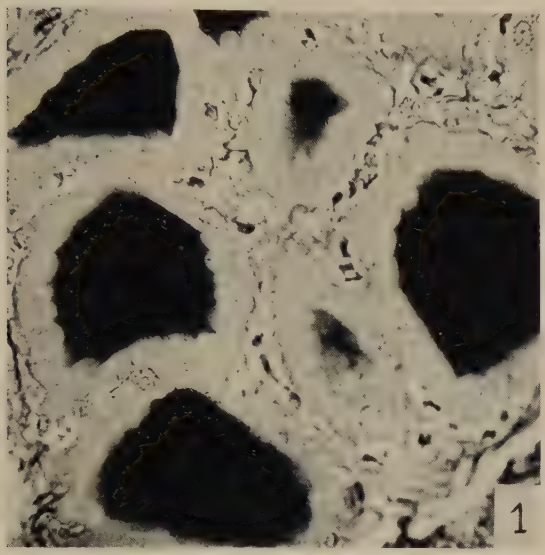
Figures 1 to 4 show photomicrographs of frozen-dried-denatured thyroid sections stained with anilin blue-orange G (60 × apoch. obj., 6 × oc., camera distance 60 cm.).

1 Thyroid of a normal control rat.

2 Thyroid of a rat 30 minutes after the injection of 3 guinea pig units of thyrotropic factor. Arrows mark sites of release of colloid droplets.

3 Thyroid of a rat 60 minutes after the injection of 1 unit of thyrotropic factor. Intracellular colloid is greatly increased and many droplets (arrows) appear to be in process of excretion.

4 Thyroid of a rat 60 minutes after the injection of 5 units of thyrotropic factor. Intracellular colloid is enormously increased.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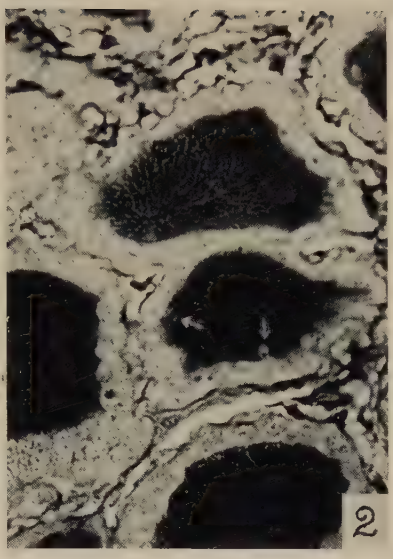
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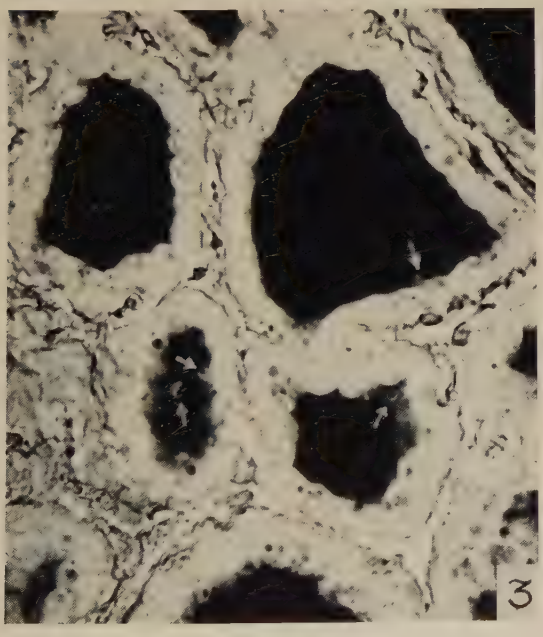
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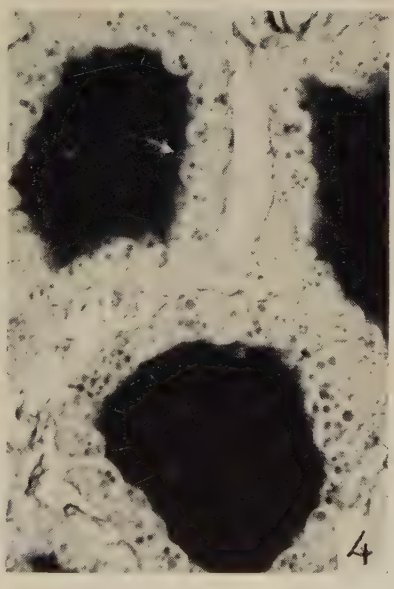
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PLATE 2

EXPLANATION OF FIGURES

Figures 5 to 9 are drawings made with the help of the camera lucida of frozen-dried-denatured sections of thyroid gland, stained with anilin blue-orange G (90 × obj., 15 × oc., table level). All cells are with the apical pole up.

5 Two thyroid cells of a rat 30 minutes after the injection of 3 units of thyrotropic factor. Both cells are excreting colloid droplets into the lumen. Three colloid droplets surrounded by a cytoplasmic film are seen in the lumen after the rupture of all connections with the cell.

6 and 7 Thyroid cells 60 minutes after the injection of 1 unit of thyrotropic factor. Two different stages of excretion are seen.

8 Thyroid cells 3 hours after the injection of 10 units of thyrotropic factor. Cytoplasm is filled with colloid. One cell has two large droplets of basal dilute colloid. No indications of excretion are present.

9 Thyroid cells 22 hours after the injection of the thyrotropic factor. One cell shows a complete inversion of its morphological polarity. c d, colloid droplets; e c, excreted colloid droplets; b c, basal colloid.

e.c.
c.d.
5
6
7
b.c.
8
c.d.
9

THE OVIDUCT AND EGG TRANSPORT IN THE ALBINO RAT [1]

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FOUR PLATES (TEN FIGURES)

INTRODUCTION

Since the function of the uterine tubes first became known, the mechanics of egg and sperm transport have been the subject of heated controversy; the fact that they conduct the female sex products in one direction (toward the uterus) and those of the male in the opposite direction, almost simultaneously, has done much to provoke interest in the problem. It is the purpose of this paper to report a study of some of the structural and functional features of the rat oviduct, where they bear upon the problem of egg transport, and to record observations of the movement of living ova within the intact fallopian tube. Reviews, which have been freely drawn upon, may be found in Westman ('26), Parker ('31), and Hartman ('39). Only work of immediate interest will be mentioned here.

Although Becker (1857) described an extensive ciliation in the mammalian oviduct and suggested its possible role in ovum transport, it was some years before the theory received any considerable support. This came from the experiments of Pinner (1880), Heil (1893), and Lode (1894), who injected suspended particles (carbon, pus-cells, and ascaris eggs) into the abdominal cavity. Their combined results showed that the injected material was carried to all parts of the abdominal

[1] This paper is a part of a dissertation presented for the degree of Doctor of Philosophy in Yale University. It is a pleasure to acknowledge the assistance and encouragement given by Prof. J. S. Nicholas, under whom the work was done.

cavity and into the oviducts; Pinner and Lode believed that a constant lymph current created by the cilia on the fimbria was responsible, while Heil suspected respiratory movements augmented by activity of abdominal organs were at least contributory factors. Lode also placed ascaris eggs on the pharyngeal epithelium of the frog and finding them carried along reasoned that tubal cilia would act in the same capacity. On the other hand, Kehrer (1863, quoted from Westman, '26) studied the excised rabbit oviduct and concluded that the cilia were unable to move rabbit ova even when placed directly upon them.

Fischel's comprehensive study of the female reproductive organs in Mus decumanus appeared in 1914 and turned attention once more to the musculature. He found considerable smooth muscle in the tubal mesentery (Musculus mesenterii tubae) and described a bundle taking origin from the longitudinal muscle of the uterine horn and connecting with the abdominal end of the oviduct and also with the hilus of the ovary — the Musculus infundibuli tubae. Fischel thought that by the action of the smooth muscle of the bursa ovarica the fluid of the periovarial space, in which are suspended the recently ovulated eggs, is compressed and, believing the ostium tubae to be the only outlet from the bursa, he assumed that the fluid would be driven toward this exit. Contraction of the musculus infundibuli tubae presumably would effect a widening of the ostium whereupon the ova would be driven into the isthmus, where tubal musculature is sufficiently developed to continue their advance by peristalsis. Fischel further suggested the possibility of a rhythmic contraction of the tubal musculature producing a sucking action (cf. Sobotta, 1895, '14), drawing the periovarial fluid into the oviduct. The cilia, he believed, form insignificant accessory means of egg transport.

Sobotta ('17) restudied the problem using guinea pigs and rabbits, which lack complete ovarial capsules. He found that activity of the smooth musculature of the adnexa during heat creates a pocket very nearly like that described for the mouse,

namely, a closed ovarial capsule. The small opening left, connecting the main portion of the peritoneal cavity with the periovarial part, is guarded by the fimbriated end of the oviduct. Here again a suction effect is assumed to be responsible for egg transport.

Westman ('26) confirmed and considerably extended our knowledge of estrous activity of the uterus and adnexa in studying these organs in the rabbit through an abdominal window. He described contractions of the mesotubarium which encloses the ovary within its folds; contraction waves in the ampulla were said to create a powerful suction which attracts free objects within the abdominal cavity. By artificially fixing the tube and ovary some distance from each other, it was shown that the suction was still effective, but considerably less so than under normal conditions.

It is, therefore, now generally held that muscular activity is the factor of primary importance in effecting the delivery of the egg from the follicle to the fallopian tube; though Westman did not study ciliary action in the rabbit, he was of the opinion that it is of negligible importance.

Advancement of the ovum through the oviduct is a problem inseparable from that just considered. It has been the subject of considerable discussion, and the principal forces invoked have again been muscular and ciliary. In 1845 Bischoff stated: "Für die bewegenden Kräfte des Eies im Eileiter halte ich die Schwingungen der Cilien des Epitheliums der Eileiter und die Contractionen des Eileiters selbst." Nearly a century later, in the most recent summary, the reviewer was forced to conclude: "At the present time . . . the preponderant opinion favors the muscular theory. There is a tendency, however, in the absence of final proof, to accede to both factors a part in the downward propulsion of the eggs" (Hartman, '39).

Sobotta has been the chief protagonist of muscular propulsion, while ciliary action has been advocated in recent years, chiefly by Grosser ('15, '18, '19); several authors have suggested a compromise (Bischoff, 1845; Parker, '30, '31).

Except for the experiments of Pinner, Kehrer, Heil, and Lode, little first-hand data are available. Again, theories have been based principally upon anatomical findings such as the presence of cilia and the presence of strong circular and longitudinal muscles. The ciliary theory lost favor when Sobotta ('14) emphasized that the uterine half of the oviduct in the mouse is devoid of ciliated cells; this has also been reported for the Muridae by Gage ('04), Schaffer ('08), Fischel ('14), Sobotta ('14), Grosser ('15), Huber ('15), Allen ('22), and Hartman ('39). Also, according to Moreaux ('13), in the rabbit ciliated cells are transformed into secretory cells just when the eggs are passing through the oviduct (see Westman, '16), a point which has been both confirmed and denied.

Sobotta ('14) has emphasized that with few exceptions, the time (generally 3 to 4 days) the ovum is in the tube in the various species is independent of (1) the length of the tube, (2) the gravid period of the animal, (3) the ultimate size or the degree of development attained. Also of interest is the fact that the rate of passage varies in different parts of the tube (Huber, '15; Andersen, '27; Lewis and Wright, '35). Opponents of the ciliary theory have claimed that this difference cannot be accounted for by ciliary action, which must everywhere be of equal rate and amplitude. However, variation in numbers of ciliated cells present in various regions of the tube and in the diameter of the tubal lumen (Grosser, '15) might well account for variations in rate. Equally fallacious is the objection of Grosser that a decrease in motility, to account for slow passage in the isthmus, cannot be presumed, for Westman ('26) has shown just such a decrease in the period following ovulation.

Concerning muscular activity, the pioneer work of Seckinger ('23), Seckinger and Corner ('23), Seckinger ('24), Seckinger and Snyder ('24 and '26) has shown that the excised oviduct exhibits contraction waves and that these vary with the ovarian cycle, being described as rapid and of varying amplitude at estrus while those of the interval are

slower and of equal amplitude. (See also Wislocki and Guttmacher, '24; Whitlaw, '33.)

Of particular interest to this investigation are the reports of von Mikulicz-Radecki ('25) and von Mikulicz-Radecki and Nahmmacher ('25, '26). Studying the rabbit oviduct in situ, contractions were observed in all non-gravid cases. These were localized constrictions which, with the contractions of longitudinal muscle, were described as producing a pendulum-like movement, the effects of which ". . . muss ein Hin- und Herschieben des Tubeninhaltes sein" (von Mikulicz-Radecki, '25). This type of movement, it is suggested, accounts for the time normally taken for the ova to pass through the oviduct. It is interesting in this connection to note that Sobotta ('17), in referring to Grosser's contention that were rhythmic muscular activity the effective force in egg transport, the time of passage would be much less than it is, suggested ". . . die Beförderung der Eier kann auch gleichsam ruckweise vor sich gehen . . ." (see also Bischoff, 1845, and Squier, '32). Whitney and Burdick ('36) also observed an "up and down" movement of eggs in estrogen-treated mice. Novak and Eisinger ('26), studying the mechanism of egg transport, sought to eliminate muscular activity as a force by painting the oviducts (of the rat) with phenol. Histological study revealed that the epithelial lining was affected but recovered more rapidly than the muscle. They found the treated side was always devoid of implantation sites. The radical nature of this experiment makes it of doubtful value. As Westman ('26) says,

> To arrive at a final conclusion as to whether one or more forces . . . assist in the ovum transit seems to be associated with very great difficulties. It would scarcely seem possible to draw any definite conclusions in this respect by studying . . . the movement of foreign bodies introduced into the tube; such gross manipulations interfere too much with a delicate biological mechanism.

Indeed, von Mikulicz-Radecki stated that this problem would not be solved until the ova themselves may be observed within

the oviduct. To accomplish this seemed very much worthwhile and not impossible.

THE MICROANATOMY OF THE OVIDUCTS

Introduction. In undertaking a study of egg transport it is essential for the investigator to be thoroughly familiar with the structural aspects of the organs in question. It so happens that this is particularly necessary for the problem at hand, as will shortly become evident. Therefore, to complement in vivo observations, a series of histological preparations was prepared including all stages of the non-pregnant and early stages of the pregnancy cycle. In all, the oviducts of thirty animals were examined.

It is conventional to divide the tube grossly into (1) the infundibulum with its fimbria, (2) the dilated ampulla, (3) a narrower isthmus, and (4) the intra-mural portion of the isthmus. In the rat these divisions are of less significance than in the larger mammals to which they were originally applied; as stated by Huber ('15) the oviduct of the rat forms ten nearly constant folds and these, as described by Sobotta (1895) for the mouse, fall into four segments. (For details see Huber, '15, pp. 261–262.)

Agduhr ('27) has, for the mouse, corrected and considerably enlarged upon earlier descriptions and although he is able, upon histological grounds, to divide the oviduct into seven segments, instead of four, as did Sobotta, he emphasizes that the significance of this is only for more exact morphological description. It has been repeatedly stated that ciliated epithelium in the Muridae is restricted to the abdominal portion of the oviduct and that "the uterine half of the tube is always devoid of ciliated cells. . ." (Hartman, '39; see also Gage, '04; Schaffer, '08; Fischel, '14; Grosser, '15; Huber, '15; Allen, '22). Agduhr ('27), however, states that in division C (abdominal portion of the isthmus), ". . . cells with true filaments (vibratile cilia with basal nodes) . . . occur, although very sparsely." These, however, are not figured nor mentioned further. It should be recalled that Sobotta's in-

dictment of the ciliary theory of egg transport was, to a marked extent, based on his belief in the total absence of ciliated cells in the greater portion of the oviducts of the Muridae; Grosser, accepting this view, yet strongly supporting the ciliary hypothesis, was forced to admit the possible assistance of muscular activity in carrying the ova through this region.

The other principal element of the tubal epithelium is the non-ciliated or secretory cell, known since Frommel (1886). The individuality of these elements (and a third one, the intercalary or "peg-cell") has long been in dispute. Allen ('22) describes a cyclic variation in these cells of the epithelium of the mouse oviduct: During proestrus and estrus

. . . the nuclei of the ciliated portion [only the "segment leading from the periovarian sac," according to Allen] are ranged in a quite regular row. As the M_1 [early metestrus] phase of the cycle advances, some of them migrate to the free ends of the cells which lose their cilia and through which these nuclei are extruded. They may retain the appearance of normal nuclei or become pycnotic before they are extruded, but when lying on the free surface of the epithelium, are shrunken and dark staining. This process reaches its height during the M_2 [late metestrus] and early D [diestrus] stages, at which time the epithelium may become greatly vacuolated.

Sobotta (1895) too refers to this extrusion process. (See also Snyder, '23, '24; Agduhr, '27; Wen, '31; and 'Espinasse, '35.)

Toward the source and the nature of the tubal fluid considerable attention has been directed, but to little avail. Schaffer ('08) strongly proposed the view that secretory cells arise from ciliated elements, describing intermediate stages. This conception has been supported by Moreaux ('13), whose studies on the rabbit led him to propose four phases in the cycle of tubal epithelium: (1) ciliation, (2) elaboration, (3) excretion, and (4) reconstruction. These are correlated with the ovarian cycle, ciliation and elaboration being associated with the maturing follicle, while excretion, he believes, falls under the control of the corpus luteum (also Courrier, '24; Courrier and Gerlinger, '22; Spaek, '23; Guerriero, '30).

Snyder's well-known studies on the fallopian tubes of the sow ('23) and the human ('24) do not give evidence of an alternation of a ciliated and non-ciliated (secretory) phase; active cilia were noted at all stages of the cycle "without apparent alteration in distribution or activity" (Snyder, '23). Periodic changes observed (and correlated with phases in corpus luteum growth) involve the height of the epithelium (more than twice as high when ova are passing through the tubes) and the morphology of the non-ciliated cells. Novak and Everett ('28) support this view. (See also Guerriero, '29 a and '29 b.) Wen ('31) supports Snyder in all essentials. 'Espinasse ('35), on the other hand, finds variations in the height of the epithelium slight and does not consider them significant. "Any cyclicity there may be," he concludes, "is clearly much smoothed out." He also finds no evidence of ciliated cells losing their cilia but, on the other hand, states that the distinction of "ciliated" and "secretory" cells has no reality.

The oviducts of the rat. It will be convenient to divide the oviduct into (1) cephalic ampulla (including the infundibulum), making up the ovarian portion of the first loop; (2) caudal ampulla, which includes the rest of loop 1 and loop 2; (3) the cephalic isthmus involving loops 3, 4, 5, and 6; (4) the caudal isthmus, including loops 7, 8, and 9. There follows the intramural portion (loop 10) within the uterine wall.

The oviduct has been examined when prepared by a variety of fixatives and stains. The ampullar regions have been examined in vitro, using the following technique: the tube is tied off between two points and the segment so isolated is carefully removed with fine scissors. Any blood is removed by rinsing in salt solution, and the tissue is then suspended in a hanging drop over a well slide. The chamber is then sealed with paraffin. It is thus possible to examine the epithelium under high magnification. The thin wall of the oviduct only slightly obscures the picture, and the ligatures maintain a nearly normal environment within. The changes which occur

rapidly when mucosa is exposed directly even to physiological solutions (Bartelmez, '40) are considerably postponed. The difficulties involved and the care necessary in the interpretation of activity of genital epithelium examined supravitally or following fixation has been emphasized by Bartelmez ('40). It seems probable that "balloon secretion," "brush border" (Agduhr, '27), and the rupturing of ciliated cells (extruding secretion of, 'Espinasse, '35) are fixation artifacts, although the bulging or rupture of the cell surface may be an indication of the accumulation of secretion within the cell.

The material on the albino rat was fixed as rapidly as possible and interpretations made with this in mind. In the rat the fimbriated margin of the infundibulum, lying within the capsule, is comprised of sixteen to eighteen folds which curve back to cover the outside of the tube for a millimeter or more (fig. 1). The folds are composed of a columnar epithelium made up of cells ranging from 25 to 30 microns in height and averaging 5 microns in width. These cells nearly always possess cilia of approximately 4 microns in length (fig. 2).

The folds of epithelium within the cephalic ampulla are again sixteen to eighteen in number and range from 60 microns to over 400 microns in height; each fold may be further subdivided into secondary folds (fig. 3). The cells are very similar, though the surface is occasionally broken by cells, frequently in pairs, which do not possess cilia. These "secretory" cells bulge very slightly but have never been seen to extend beyond the tips of the cilia. The so-called "club-cells," described by Agduhr ('27) and others as being present in "great numbers" and frequently to be seen lying free in the lumen are rare. Indeed, one of the most striking differences noted is this rarity of the phenomenon of "nuclear extrusion" (Sobotta, 1895; Allen, '22) or "cellular extrusion" (Wen, '31; 'Espinasse, '35). Occasionally small wedge-shaped nonciliated elements are seen superficially located, apparently pushed up between the ciliated cells, but rarely is there an

indication of these being ejected, and the picture of a rough epithelial surface dotted at frequent intervals with "extruded" elements, so typical of the mouse, is totally lacking in the rat (figs. 1, 2, and 3).

The caudal ampulla passes rather more gradually into the isthmus than is the case in the mouse. The folds are lower and fewer in number. In the mouse (according to Agduhr, '27) the epithelium is mostly of the "secretionary type (balloon secretion)" with numerous cells showing vibratile cilia. This region in the rat, comparable so far as may be judged from description and figures, is still chiefly composed of ciliated elements, though cells devoid of cilia are more frequently seen than in the preceding section.

The transition to the isthmus is, again, gradual; the layer of circular muscle becomes progressively well developed, reaching a thickness of 100 microns in the caudal isthmus as compared to 30 to 40 microns for the caudal ampulla. The isthmus makes up the greater portion of the tube, and the folds, longitudinal at either end, become transversely disposed throughout this portion. In this region Agduhr ('27) states for the mouse, "Numerous epithelial cells show balloon-secretion. Epithelial cells with true filaments (vibratile cilia with basal nodes) also occur, although very sparsely."

Examination of properly prepared sections of the isthmus of the oviduct under sufficient magnification shows that much of the epithelium is made up of cells bearing typical cilia arising from a double row of basal granules, as do those of the cells of the ampulla. In some cells the granules are only faintly visible, and in others not at all (cf. figs. 4 and 6).[2]

[2] Since this writing a paper has come to hand in which the author correctly observes that "Dans les troisième et quatrième segments de la trompe, les cellules épithéliales ciliées vibratiles se retrouvent dans les culs-de-sac formés par les replis de la muqueuse, mais elles y sont peu nombreuses, cubiques et reconnaissables par les corpuscules basaux de leurs cils et la coloration intense des cils; ceux-ci sont parfois incurvés en crochet à leur extrémité" (H. Lams, 1923, L'Épithélium de l'oviducte pendant le passage de l'oeuf chez quelques mammifères (rat, cobaye, souris). C. R. d. l'Assoc. Anat., vol. 18, pp. 281–286).

Ciliated cells also are found in the caudal isthmus. Here the cells seen in sections appear to be in isolated crypts (figs. 7 and 8); actually they lie in grooves formed by the folded epithelium, as may be determined by examining a portion of the oviduct in a hanging drop. There is a tendency for the clearly ciliated elements to be confined to these grooves. This is not, however, invariably the case; in some instances they extend up the side of the folds, and more rarely are found on the tip. Over what length these ciliated bands maintain their integrity and whether they anastomose with one another has not been determined. Ciliated cells become increasingly rare as the uterus is approached and have not been found in the immediate region of the utero-tubal junction.

In general, two to six contiguous cells with cilia arising from prominent basal granules form the floor of the groove. Certain neighboring cells frequently show cilia, but the basal granules may be indistinct or absent. Still others show on their surface faint strands which are clearly cilia, but no basal granules are present. These can hardly be the "brush border" artifact of Bartelmez ('40) due, according to this author, "to the formation of threads of secretion extending from the surface," for they are quite uniform in length and of the same construction as cilia with definite basal granules. Nor can they be the "buschelformigen Faden" mentioned by Schaffer ('08). Frequently cells which obviously bear cilia are found to be considerably enlarged and with cytoplasm staining somewhat lighter than that of the surrounding cells; their distal end may bulge toward the lumen (fig. 5). Further, in some cases cells which have what appear to be "degenerate" cilia exhibit "bleb formation" or "balloon secretion" similar to that described by Agduhr ('27) for non-ciliated cells. In some instances these "extruded" blebs bear what appear to be the remnants of cilia (fig. 5). If we assume that bleb formation is evidence of intracellular stores of secretion, these findings support the contention of 'Espinasse ('35) that the distinction between "secretory" and "ciliated" elements has no basis. On the other hand, it appears that cilia may be lost

(contra Wen, '31; 'Espinasse, '35). Considerably more elaborate techniques must be employed before this problem can be settled. However, the point to be emphasized is that ciliated cells, in which the organelles arise from prominent basal bodies, occur in bands or in isolated pockets practically throughout the oviduct of the albino rat; "grosse zusammenhängende Strecken der Tube" (Schaffer, '08) are not completely devoid of ciliated elements.

It has not been possible to demonstrate clearly any cyclic changes in the histological picture of the oviduct. Changes in the amount of fluid in the ampulla are clearly evident, but the source of this fluid has yet to be certainly established. It is very possible that recurring changes which do take place in the epithelium are obscured by the shortness of the cycle in the rat.

EGG TRANSPORT IN THE RAT

Methods. There are serious objections to all methods used in studying the uterine tubes in mammals. In vitro studies are unsatisfactory for obvious reasons: the nerve and blood supplies are usually severed and the effects of perfusion fluid may be considerable. Abdominal windows have some advantages, but the view is frequently totally obscured by moisture condensation (Nicholas, '34) and other abdominal organs, and only the grosser movements are visible; the actual effect of these movements on the ova is impossible to observe. The probability of irritation from an abdominal window producing non-physiological activity in the viscera has been emphasized by von Mikulicz-Radecki and Nahmmacher ('26). A laparotomy under anesthesia is sometimes considered the least reliable technique. However, it has many advantages, the chief of which is that detailed observations are possible. It seemed probable that by using the transillumination technique of Knisely ('36) the eggs themselves might be followed within the oviduct (see Squier, '32; Lewis and Wright, '35; Burdick and Whitney, '41).

Sodium amytal anesthesia (Nicholas and Barron, '32) was used. It is known that even in full anesthesia smooth muscle depression is much less than with ether; uterine musculature is particularly resistant to the drug, and its response to pituitary secretion remains normal. Entrance into the abdominal cavity was made either through a ventral incision or by one just lateral to the axial back muscles and immediately below the last rib, following the technique of Nicholas and Rudnick ('33). The instrument used for transillumination was a simple modification of that described by Knisely ('36). This instrument was attached to an Emerson micromanipulator so that it was possible to vary the position of the light with great exactness. For detailed observation 20X oculars and 50X objectives were used. Although it is quite possible, if respiratory obstruction does not become acute, to maintain an animal under observable conditions for the better part of a day, observations over periods of more than a few hours were considered of questionable value and were not generally made; it was thought safer to close the wound and allow the animal to recover from the anesthesia between observations when these were extended.

Observations. Observations using this technique include those made on oviducts containing artificially introduced material and those containing fertilized and unfertilized ova.

When the exposed organs are viewed as a whole under low magnification during the onset of estrus, rhythmic contractions are evident both in the tubes and the uterus. Not only are the uterine horn and adnexa being moved anteriorly and posteriorly within the body cavity by activity of the uterine and suspensory ligament musculature, but waves intrinsic to the oviduct and to the uterus are clearly discernible The fluid distending the uterine cornu is churned backward and forward by peristaltic and antiperistaltic waves. If copulation has taken place this fluid is filled with sperm, and the muscular activity delivers these rapidly to the utero-tubal junction.

Activity of the oviduct is also marked, but it is extremely difficult to determine the exact nature of the contraction waves.

They are more evident in the isthmus than in the ampulla and appear to originate and die away at various separate points along the tube, involving one or two loops alone, these then stimulating a neighboring region (cf. Westman, '26; von Mikulicz-Radecki, '25). The peak of activity is reached at about the time of ovulation and involves all parts of the uterus and adnexa. There follows a marked diminution of general activity as early metestrus approaches, though the tubal contractions remain.

Of more moment is the activity seen under high magnification with the aid of transillumination. Only a limited section of the oviduct may conveniently be kept under prolonged observation, so that the type of muscular activity as such cannot be judged; it must be deduced from the resultant effects. The cephalic loops of the oviduct are distended with fluid and the epithelial folds thus become widely separated. The epithelium between the folds is typically only one cell in thickness. Since the musculature and stroma in this region are weakly developed, it is possible to see through the thin wall of the tube (fig. 9). The details of blood supply are clearly shown, a single large vessel always being particularly evident in the mucosa of each fold. Indications are that cyclic changes in the vascular architecture occur in the tube as well as in the uterus (Bacsich and Wyburn, '40). The active cilia are clearly visible; all beat toward the uterus. When this ciliated epithelium is viewed by transillumination one can readily understand why Sobotta (1895), in referring to it, said, "Es ist . . . das schönste Flimmerepithel, das ich je bei Säugern gesehen habe." The individual cilium is approximately 4 microns in length and arises from a pair of basal bodies which readily stain with janus green, as also do supranuclear and infranuclear intracellular elements.

If ovulation has occurred, the second loop of the oviduct almost invariably contains the ova, each surrounded by its cumulus oophorus. These cell masses are typically close together, not infrequently forming one large clump. From the center of each cumulus, which appears greyish in the

transmitted light, the individual ovum glistens brightly. Ova in the dilated cephalic loops are freely suspended in the tubal fluid (fig. 9). Later, as the group progresses through other loops which do not show the marked dilation of the second segment, the ova become spaced one behind the other (fig. 10) and eventually lose their corona cells.

The movement of material contained within the oviduct was first observed with injected matter — ascaris eggs previously stained, and the vital dye, janus green. The ascaris eggs as a rule were not of as much service as the dye alone. Using the normal opening into the periovarial space (Alden, '42 a) it is possible to introduce foreign material into the periovarial space without greatly upsetting normal conditions, as would be the case if the capsule were punctured. By using a specially constructed pipette it is possible to introduce material into any part of the periovarial space or into the ostium of the oviduct itself. Where ascaris eggs were used, they were taken up in the pipette with as little solution as possible and introduced into the capsular opening. Since the periovarial sac is quite distended with its own fluid, it was frequently necessary to draw off a small quantity to prevent the introduced material from being ejected as the pipette was withdrawn.

The janus green proved suitable because the fluid was more easily introduced than the particulate material and it soon formed many small clumps, a few closely simulating an egg with its corona as far as size was concerned. When a few drops of dye were introduced into the periovarial space close to the ostium of the oviduct, the material was rapidly passed through the infundibulum and in a matter of seconds was found within the first loop of the oviduct. It could readily be seen that the clump (of nearly any size able to enter) was carried along by the visible, rapidly beating cilia.

To examine the fimbria surrounding the ostium, the capsule was resected in several animals. The fimbriated end is somewhat expanded during estrus as compared to its condition during the interval. This is evident in the living animal

and in histological preparations (cf. Agduhr, '27). Whether this is due to hyperaemia of the tissues or to the Musculus infundibulum tubae (Fischel) could not be ascertained, but it is believed likely that both play a part. Particles (some considerably heavier than an ovum with its cumulus oophorus) placed on the fimbria facing the capsule were readily carried by cilia beating towards the ostium abdominale. The sum total of all observations made has convinced the observer that the action of the cilia of this region of the oviduct is sufficiently powerful to account for the movement of the eggs from the periovarial space into and through the first loop of the oviduct.

Once within the second loop of the oviduct the situation changes. Although ciliary action supplies a constant force tending to drive the tubal contents toward the isthmus, a tonic constriction at the uterine end of the loop prevents its passage. Within this confined segment the contents were seen to be driven forward, then backward. Small particles breaking off or cells normally lying free in the lumen were seen to be swept rapidly along by the ciliary current, only to be hurled backward or further forward, as the case may be, by muscular activity (cf. von Mikulicz-Radecki and Nahmmacher, '25).

When the ova themselves were observed the picture was similar; if anything they were more localized in their movements than the dye mass, the constriction preventing their advancement being especially prominent. When, inexplicably, this contraction was released, they were driven (or sucked?) into the neighboring loop. If a constriction occurred to prevent their return, they remained in the advanced position; if not, they returned to the loop just vacated. Rarely did the extent of the pendulum-like movement exceed a single loop of the oviduct. Thus advancement was hesistant, a segment at a time.

As the ova separate from the cumulus cells following sperm contact their advancement may no longer be as a group. Individual ova may attain a more caudal loop, while others are held in its cephalic neighbor. It is not common for the

ova to be widely distributed, however, and seldom are they separated by more than one loop.

Observation of ova beyond the caudal ampulla is difficult due to the well-developed musculature of the isthmus. If a small quantity of vital dye is introduced into the oviduct immediately after ovulation, the general distribution of the ova may be followed for the first 36 to 48 hours. In one instance the movement was followed through the first and second loop during the 8 or 10 hours after estimated ovulation time. The abdomen was then closed and the animal allowed to recover from the anesthetic. At about 50 hours post-ovulation time it was again examined and the dye particles were visible in the fifth loop, the region in which two- to four-celled ova are normally found at this time.

Evidence at hand, both that from in vivo studies and from histological preparations fits, in general, with Huber's ('15) data on the rate of passage through various regions of the tube. The segmenting ova reach the caudal isthmus some hours before actual entry into the uterus, which seems to occur between 95 and 100 hours after ovulation. They have been found to reach this portion of the oviduct as early as 60 hours, but 72 to 80 hours is the more usual time. There is evidence that the entry of the eggs into the uterus is controlled by the musculature of the utero-tubal junction.

Discussion. It is clear that much of the isthmus of the rat oviduct possesses an epithelium which is, in considerable part, made up of cells bearing vibratile cilia. Furthermore the only region of the tube wholly devoid of ciliated elements is a very short section of the intramural oviduct in the immediate region of the utero-tubal junction. The histological picture indicates the rarity of nuclear (or cellular) extrusion and on the whole throws doubt on the validity of the distinction between secretory and ciliated elements.

The action of all cilia observed in the living state in the intact oviduct was such as to produce an abovarian current and no "temporary longitudinal compartments" with proovarian currents, such as have been reported for the rabbit

(Parker, '30, '31), were noted. In his discussion of the carrying power of cilia, Parker ('31) lays considerable stress upon the need of the object in question to "be pressed with some vigor against the cilia" and states that the oviduct is constructed to assure just such a relationship between the egg and the ciliated epithelium. He pictures the egg as moving in the compartments formed by the epithelial folds and since these compartments are slightly smaller than the ovum, the latter press against the tubal folds, thus assuring intimate contact with the cilia. "In fact," he concludes, "the tube possesses a structure which in form and proportions is most admirably adapted to the propulsion of the egg by cilia" (Parker, '31).

In the rat conditions are very different. The dilated ampulla provides a lumen many times the diameter of the egg even with its satellite follicle cells (fig. 9; see also Burdick and Whitney, '41, fig. 2); indeed, six or eight ova with their accompanying corona cells may be clumped together in a single mass and yet not occlude the tubal lumen. Even in the more posterior loops the ova may still be freely suspended in the tubal lumen (fig. 10), and only in the caudal isthmus are the ova firmly in contact with the epithelium. And yet the action of the cilia, at least in the ampulla, is sufficient to move the tubal fluid with the ova suspended in it. Hence, the position of the egg in the tubal lumen and the intimacy of its contact with the epithelium vary strikingly in different parts of the oviduct.

The evidence supplied by transillumination of the oviduct indicates beyond doubt that the ciliation of the infundibulum and the ampulla is sufficient to carry the egg with its corona of follicle cells; indeed, the author is convinced that ciliary action is primarily responsible for the transport of the egg from the capsule to the oviduct. It is of course impossible to state categorically that in the movement of the material additional forces are not active such as the suction postulated by Sobotta, or that resulting from compression of periovarial fluid, as suggested by Fischel. It can only be said that the

nature of the movement observed appears to be caused by cilia alone. It is certain that cilia are perfectly capable of transporting the eggs.

Within the infundibulum the ova are carried rapidly forward (toward the uterus) by the ciliary current and under given circumstances would undoubtedly be quickly transported through the ampullar loops and into the isthmus. Here one might expect the movement of the egg to be less rapid, due to weaker ciliation, and to become progressively slower as the uterus was approached. Such an explanation would account for the observed variation in the rate of passage in the different parts of the tube (Huber, '15; Andersen, '27; et al.) as well as the total time taken to reach the uterus. However, observation by transillumination suggests that, while ciliary action is the primary force in effecting entrance of the egg into and through the cephalic ampulla, action of the tubal musculature is the controlling factor governing further advancement. The ciliary current exerts a constant force tending to propel the egg toward the uterus, but this action is modified — frequently counteracted — by muscular contraction. The result of this muscular action is such as to produce a movement of the tubal contents apparently identical to that reported by von Mikulicz-Radecki and Nahmmacher ('25) for the rabbit.

The point of origin of the force effecting this "Pendelumbewegung" (von Mikulicz-Radecki, '25) is difficult to determine; it definitely does not seem to be of local origin. The smooth muscle of this region is hardly sufficiently developed to produce the activity in question, but, more to the point, it is not evident to the observer. Although slight ampullar contractions do occur, they cannot be correlated with the rapid backward and forward movement of the contained particles. This would suggest that the force involved is produced by the activity of the caudal ampulla and, more especially, the isthmus (cf. Whitney and Burdick, '36) which is particularly active at this time. Although local rhythmic contractions of circular muscle are not evident, there is an indication that longitudinal

fibers, few as they are, may be active in producing the to-and-fro movement (cf. von Mikulicz-Radecki, '25). Unfortunately, it has not been possible to study tubal contractions by the kymograph method.

The significance of this rather extraordinary method of egg transport — involving a violent forward and backward movement of the tubal contents — is rather hard to imagine and the method of progression difficult to analyze. There seems to be a gradual release of the caudal constriction and an advancement of the cephalic one. In any event, the rhythmic forward and backward propulsion of the tubal contents continues from one loop to the next. It is evident that the to-and-fro movement may account for the delay which Grosser found difficult to explain on the basis of purely muscular peristalsis. It also may be reconciled with some reports of the effects of hormones on egg transport — the tube-locking of ova with estrin injections of proper dosage (Burdick and Pincus, '35; Pincus and Kirsch, '36; Whitney and Burdick, '36) and the acceleration of passage in cases of superovulation (Wislocki and Snyder, '33). As has been pointed out (Burdick and Pincus, '35) such facts tempt one to infer that hormones control egg transport, estrogens inhibiting egg passage, while progesterone accelerates it (see Alden, '42 b). The implication would then be that active tubal musculature delays egg transport, while a quiescent musculature is conducive to rapid passage through the oviduct (Kok, '26). Whether ciliary action in a quiescent tube would deliver the ova in a shorter time is difficult to ascertain. It is quite certain that in the rat the developing ova reach the utero-tubal junction several hours before actual entry into the uterus. Long and Evans ('22) noted that tubal ova of unmated rats (animals without functional corpora lutea) never reach the uterus. Many results, including unpublished ones accruing from this study,[3] indicate that the utero-tubal junction, responding to nervous or hormonal action, is the controlling factor governing passage of sex products from oviduct to uterus and vice

[3] These will be reported at a later date.

versa. That other regions of the tubal musculature may be subject to hormonal action and so affect movement of the tubal contents, however, is indicated by considerable work, particularly the recent results of Burdick, Emerson and Whitney ('40), who report ova being held in the ovarian third of the tube for 6 days.

SUMMARY

1. A restudy has been made of the microanatomy of the rat oviduct. Observations by transillumination of movements of ova within the oviduct and of tubal contractions suggest a possible explanation of the mechanics and rate of egg transport.

2. The isthmus of the rat oviduct, usually described as being devoid of cilia, possesses ciliated elements practically throughout its length. In the more caudal portion of the tube the cells bearing cilia tend to be restricted to the base of longitudinal grooves formed by the folds of the epithelium.

3. There is some histological evidence suggesting that: (a) an alternate degeneration and genesis of cilia may occur in the isthmus; (b) ciliated elements probably secrete.

4. Direct observation of movements of ova within the oviduct suggests that: (a) entrance of the egg into the oviduct is effected primarily by the action of cilia; (b) further progress is governed by muscular contraction which effects a backward and forward movement of the tubal contents and results in a hesitant advance of the ovum, one segment at a time.

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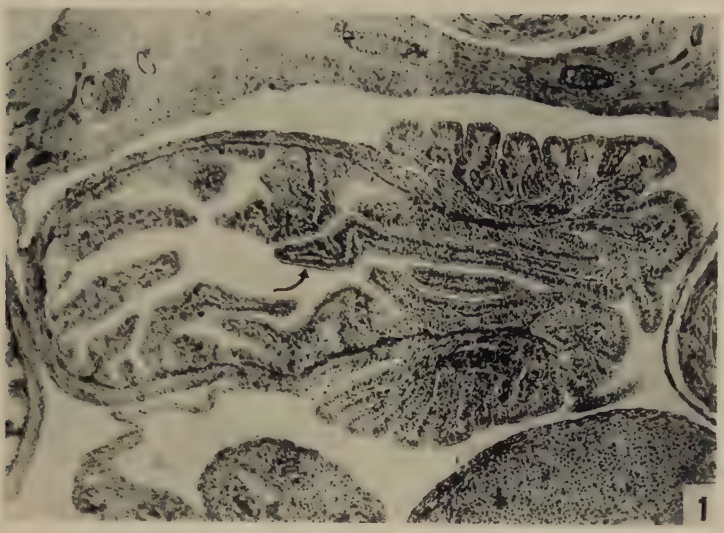
PLATE 1

EXPLANATION OF FIGURES

1 A section through the intracapsular portion of the oviduct showing the fimbriated margin of the infundibulum. Note that the epithelium curves back to cover the outside of the tube for a millimeter or more, forming a ciliated collar about the ostium abdominale. Magnification approximately × 45.

2 An area of epithelium from the region indicated by the arrow in figure 1. Note the evenness of the epithelial surface and the well developed cilia arising from a double row of basal granules. Magnification approximately × 1000.

3 A section through the third loop of the oviduct where the ampulla grades into the isthmus. Note the even character of the epithelial surface, the regular arrangement of the nuclei, and the absence of cellular or nuclear extrusion. The animal was in early metestrus. The ciliated surface is only rarely interrupted by non-ciliated elements. Magnification approximately × 75.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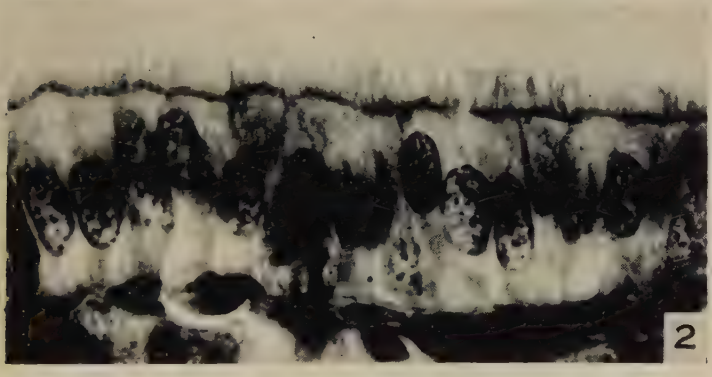
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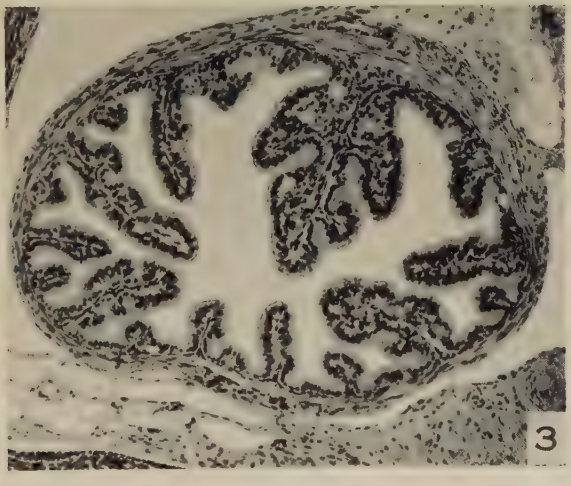
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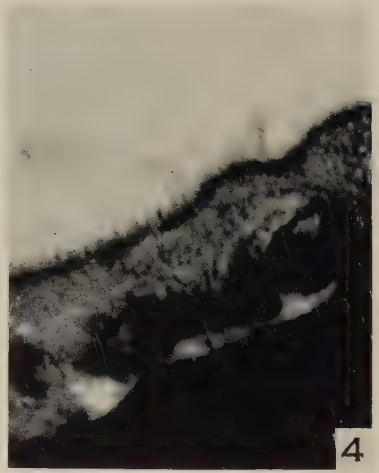
PLATE 2

EXPLANATION OF FIGURES

4 A section of epithelium from the isthmus. Note that the cells bear cilia which arise from basal granules. Magnification approximately × 2500.

5 A section of epithelium from the same area as in figure 4 showing "extrusion blebs." Note the faint ciliation and absence of basal granules. Magnification approximately × 2500.

6 A section of epithelium from the same area as in figure 4. Note that cilia are present but that basal granules are faint or absent. Magnification approximately × 2500.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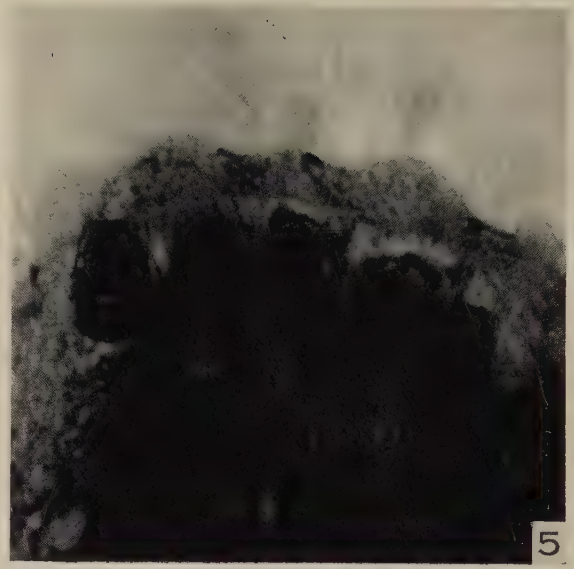
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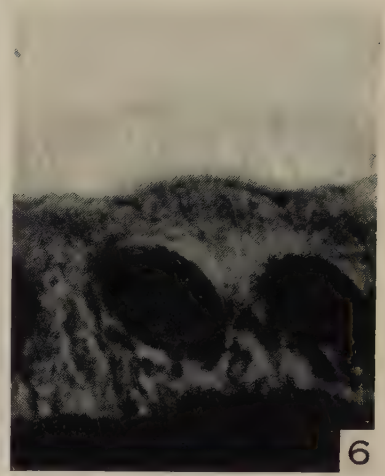
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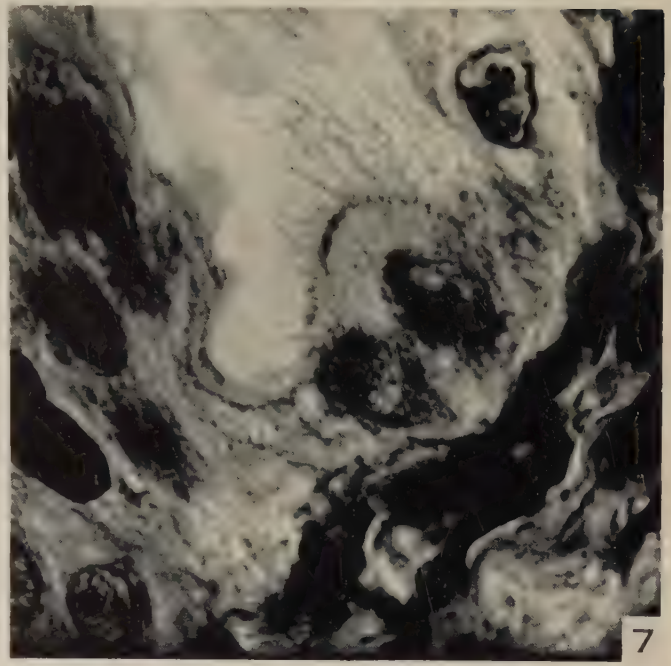
PLATE 3

EXPLANATION OF FIGURES

7 and 8 Ciliated crypts in the caudal isthmus approximately 2 mm. from the utero-tubal junction. Note that the pair of cells which form the floor of the crypt possess cilia which arise from basal granules. Magnification approximately $\times$ 3000.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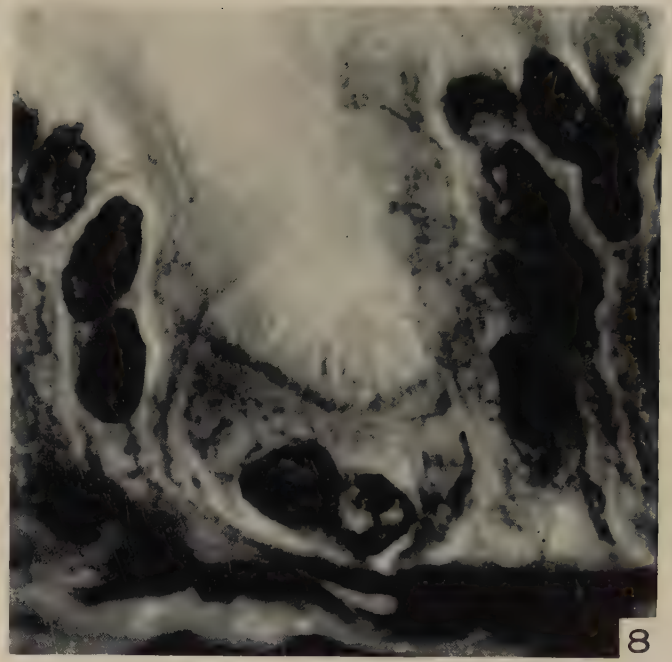
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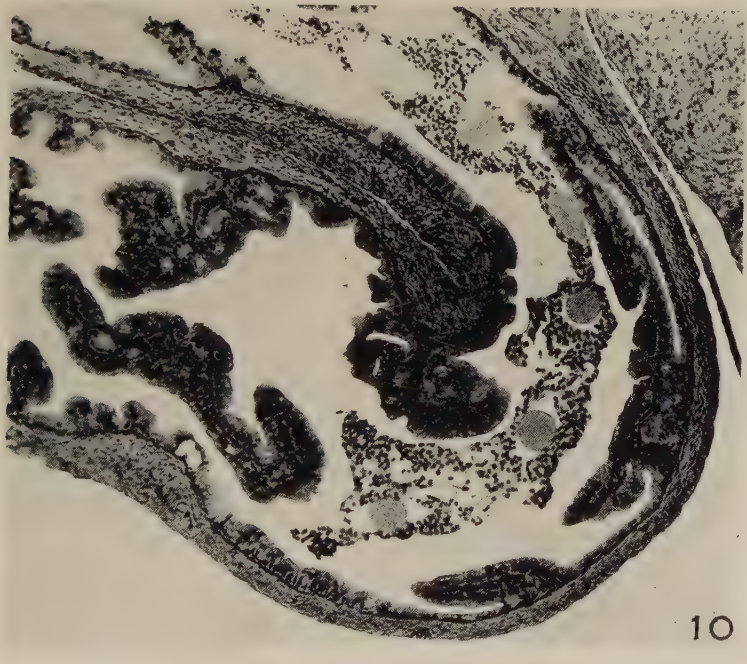
PLATE 4

EXPLANATION OF FIGURES

9 A section through the fluid-filled loop of the ampulla showing the manner in which the egg is suspended freely within the lumen of the oviduct. Note the relatively thin wall of the oviduct. It is in this region that the ova are most easily observed by transillumination. Magnification approximately × 75.

10 A longitudinal section of the oviduct showing the disposition of five ova in the cephalic isthmus. Note that here there is little or no intimate contact of the ova, with their dispersing corona cells, and the tubal epithelium. Magnification approximately × 75.

9



10

A STUDY OF THE HEPATIC-CELL MITOCHONDRIA IN THE FATTY LIVER PRODUCED BY A HIGH-SUGAR DIET

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TWO PLATES (TWELVE FIGURES)

That the mitochondria are the cell inclusions actively associated with the laying down of fat in liver cells is the opinion of several cytologists. Kater and Smith ('32) describe the fat as being formed within vesiculated mitochondria, while Noël and Pallot ('34) consider it elaborated on the surface of enlarged mitochondria. Others, however, have not found what they consider a direct relationship between mitochondria and fat droplets within the liver cells (MacCardle, '37; McCurdy, '39; and Steffens, '41). Because of these conflicting views, it was thought that it might be profitable to restudy the problem using an essentially non-pathological method of producing a fatty liver.

For this purpose, the high-sugar diet used by Barrett, Best and Ridout ('38) was adopted because it produces a marked rise in liver fat in the mouse and because they have studied the process biochemically. In order to ascertain the origin of liver fat, they first labelled the body fat with deuterium. Since the liver fat, after the diet, contained little deuterium, this fat was assumed to have been formed by conversion from the sugar in the liver itself. A secondary accumulation of fat resulted when the diet was followed by a 2-day fast, and the fat in this case was shown to be derived from the fat of the

body depots, since it contained the same proportion of deuterium.

I wish to thank Prof. J. Walter Wilson for the suggestion of this problem and for his aid and helpful criticisms during the course of the investigation.

MATERIALS AND METHODS

The mice used were raised in the laboratory from several stocks. After weaning they were maintained on a diet of Kasco Dog Chow, supplemented twice weekly with milk and weekly with carrot. At approximately 2½ months of age they were placed on the high-sugar diet devised by Barrett, Best and Ridout ('38):

Sucrose	76 parts
Agar	15 parts
Dried extracted beef[1]	5 parts
McCollum's salt mixture no. 185	4 parts
Brewers' yeast — Harris	4 parts
Cod liver oil	1 drop daily to each mouse

[1] Kindly supplied by Valentine's Meat Juice Company, Richmond, Virginia.

The animals were killed by a blow on the head and bled. Several pieces of liver from the left-central lobe were immediately fixed in 10% neutralized formalin in a 0.9% NaCl solution.

For mitochondrial preparations, a modified Regaud technique was followed. A piece of liver, after fixation in formalin for 24 hours or more, was post-chromed in 3% $K_2Cr_2O_7$ for 3 days at 37.5°C., washed for 24 hours in running water, imbedded in 56–58°C. paraffin and sectioned at 3 micra. These preparations were stained by the long iron-alum hematoxylin technique of Heidenhain. This simplified Regaud technique is an apparently satisfactory substitute for the original. Schiller ('30) has shown that 4% formaldehyde in an isotonic salt solution fixes the whole organ satisfactorily and preserves the mitochondria. The post-chroming makes the mitochondria insoluble in the dehydrating agents.

For fat preparations, a block of liver, fixed in formalin for at least 48 hours, was imbedded in gelatin (Zwemer, '33) and sectioned on the freezing microtome at 10 micra. The sections were fixed on the slide with 1% gelatin and stained with sudan IV and Delafield's hematoxylin.

In each of the three studies described below, animals of both sexes were killed each time, primarily to have two or more examples for each treatment and also to detect any sex difference, if present. (1) To obtain a description of the development of the fatty liver, animals were killed from 24 hours to 6 weeks after being placed on the diet (forty mice). Each of these mice was deprived of food at 9:00 A.M. and killed 6 hours later so that it would not be actively absorbing food from the intestine. Observations on the normal diurnal cycle made during the course of this investigation on the mouse and by Deuel et al. ('38) on the rat indicate that such rodents eat mainly at night, with the liver storage products reaching a maximum in the early morning and a minimum in the afternoon. (2) For a study of the effect of inanition on the dietary fatty liver, animals were maintained on the diet for 1 to 4 weeks, then starved for 48 hours before sacrifice (nine mice). (3) To obtain a description of recovery from the fatty-liver condition, animals were fed the high-carbohydrate diet for 2 weeks, then restored to the stock diet and killed after 1 to 4 weeks of recovery (eighteen mice). They were removed from food in the morning and killed in the afternoon like those in Group One.

OBSERVATIONS

Control animals, fasted 6 hours. In the normal mouse, fed Kasco Dog Chow and killed in the afternoon after a 6-hour fast, the liver cells contain a few small droplets of fat which stain lightly with sudan IV. The appearance of the mitochondrial preparations agrees essentially with the descriptions of Noël ('23) and Kater ('33). Under low power, the liver lobule has a zoned appearance because a few rows of cells around the central vein contain very fine mitochondria

and hence stain less intensely with the hematoxylin than the cells in the rest of the lobule (fig. 1). The mitochondria of the central zone are extremely sparse, fine, long, beaded filaments. In the middle zone, the beaded filaments are somewhat thicker and extremely crowded in the cells. In the outer part of the middle zone, the filaments are shorter, with a gradual transition to the peripheral zone (which is the few rows of cells adjacent to the portal canal), where the mitochondria are in the form of thick, beaded rods, spheres, and occasional bulbs and vesicles.

Group One: animals on the high-carbohydrate diet. All of the animals fed the high-sugar diet appeared healthy in all respects at the time of sacrifice, and their livers were of the usual color and texture.

Fat is rapidly accumulated in the livers of animals on the diet. In those cells immediately surrounding the central vein, the droplets are fine, almost dustlike, in all preparations of this series. By the fourth day, droplets in the cells outside the central zone are large and crowded and stain a bright orange-red with sudan IV. With longer treatment, the fat droplets become larger, often confluent. In both sexes, there is a gradual reduction in the histologically preserved fat after the second week, and it is lost almost completely in the two or three rows of cells immediately surrounding the central vein. This reduction in the amount of sudanophil fat is more conspicuous in the males than in the females. A considerable amount of fat is still present, however, in the livers of animals killed even at the termination of the experiment, 6 weeks after beginning the diet. By this time, other vacuoles, apparently watery, appear in the peripheral-zone cells.

Particularly during the first week of the diet, certain scattered cells are found which contain fat which stains crimson rather than the lighter orange-red. Such cells will hereafter be referred to as "crimson" cells (fig. 10).

The mitochondria in those cells adjacent to the central vein have the form of fine, long, tangled, beaded filaments in all preparations, no matter how long the treatment, and they tend

to become very sparse. This description is a modification of my original one (Deane, '41), in which I said that the mitochondria of the whole central zone eventually become enspherulated: enspherulation stops one or two rows of cells from the central vein. The mitochondria of the cells of the middle zone of the lobule tend to be shorter, and they often exhibit a rather conspicuous orientation perpendicular to the sinusoid (fig. 2). In the males, by the end of the first week, the mitochondria are short, chunky rods, spheres and vesicles, but after the end of the second week they gradually become more elongated beaded rods and filaments of about normal dimensions. In the females, on the other hand, the mitochondria of the middle zone are shortened and swollen from the second day of the experiment until the end of the second week. In this sex, also, the mitochondria of this zone become more elongated beaded filaments in animals killed after 3 or more weeks of the diet. The mitochondria of the cells in the peripheral zone of the lobule are spherical from the beginning of treatment in the females (fig. 3) and by the end of a week in the males. By the end of the second week they are reduced in diameter (fig. 4) and by the sixth week even elongated.

The cells of animals killed 2 or more weeks after the beginning of the diet are filled with large, irregular vacuoles which occur especially in the peripheral half of the lobule. The mitochondria are by necessity crowded into the cytoplasmic network and appear "clumped" and are less easily differentiated from the cytoplasm, which retains more hematoxylin than usual (fig. 5).

During the first 10 days of the treatment there is an apparent tendency for some of the cells to become "black," that is, to stain intensely with hematoxylin. In some of these it is possible to make out an indication of spherical mitochondria in the cytoplasm (fig. 9); in others the cytoplasm appears homogeneous (fig. 6). In a few isolated cases there are many of these cells in the portal zone. During this early period in the diet, furthermore, scattered cells may contain

huge, spherical mitochondria. Other cells, often near the veins, have no apparent mitochondria, being homogeneously lightly stained or "clear" (fig. 7). After a 2-week feeding period, however, the cells of a given zone are quite uniform in appearance, and the "black" and "clear" cells only rarely occur.

Control animals, fasted 48 hours. After a 2-day starvation, a mouse is generally weak and feels cool to the touch. The liver is light colored, friable, and has a somewhat nodular appearance.

The liver cells are generally very vacuolated, occasionally having a honey-combed appearance, like adrenal cortical cells. Lightly staining fat is usually distributed throughout the lobule but may be concenterated in the central half (fig. 11). Fat droplets are to be seen among the blood cells in the veins in many preparations. In mitochondrial preparations, the central zone is conspicuously light under low power. The mitochondria have the form of pale, beaded filaments or small granules in the cells of this zone in all preparations. In the cells of the middle zone they are stubby rods and spheres, frequently vesiculated. In the peripheral-zone cells, the mitochondria are thick, swollen rods and spheres, frequently vesiculated, and often with irregular diameters. "Black" cells are fairly common (fig. 6).

Group Two: animals on diet, starved 48 hours. Liver sections of animals fasted 2 days after various lengths of time on the high-carbohydrate diet appear vacuolated and sometimes honey-combed. Medium sized fat droplets fill most of the cells in the preparations of the first 2 weeks. When zonation occurs, the fat is limited to the central part of the lobule. In some preparations there are vacuoles which do not contain fat (fig. 12). In the male mice the fat is somewhat reduced in the preparations of the third and fourth weeks of the experiment just as it is in the males of Group One. In several animals "crimson" cells are scattered in the lobule. Fat globules can be seen among the blood cells in the veins.

In both sexes, the mitochondria of the central zone may be well stained granules or may be only lightly stained or may be apparently absent (fig. 7). For the first 2 weeks, the mitochondria of the cells of the middle zone are short, swollen rods and beads, becoming finer beaded rods later. Those of the portal zone are spherical, sometimes fused, during the first 2 weeks and become beaded filaments during the third and fourth weeks. Those of the females appear somewhat more swollen and of more uneven diameter in the two outer zones during the first 2 weeks than do those of the males. "Black" and "clear" cells are scattered throughout the lobule.

Group Three: animals recovering after 2 weeks on the diet. There is a rapid and complete recovery from the fatty-liver condition in the animals restored to the stock diet of Kasco Dog Chow after 2 weeks on the high-sugar diet. Fat globules persist for a few days, particularly in the central portion of the lobule, but have disappeared by the end of a week. Later, however, a few fine droplets do reappear in the liver cells, similar to those in the control animals.

In all of these animals, the mitochondria of the central zone are fine, discrete beaded filaments (fig. 8). Those of the middle zone are likewise beaded filaments, broader and more crowded than those of the central zone. The mitochondria of the peripheral-zone cells are discrete and crowded short, beaded rods and spheres in all preparations, male and female (fig. 9), except in the females during the first day or two, when they are somewhat swollen. For the first week, "black" and "clear" cells are scattered in the lobule, and non-fatty vacuoles are present. With the subsequent reduced vacuolation and the less swollen appearance of the mitochondria, the liver lobule and cells appear essentially normal after about a week of recovery.

DISCUSSION

This investigation was designed to study the relation of the hepatic-cell mitochondria to the deposition of fat in the liver.

Free fat can be demonstrated in liver cells under many circumstances, for example, when there is an excess of fat in the diet, when it is converted from sugar in a high-carbohydrate diet, when it is mobilized from the body depots, when there is a vitamin-B complex imbalance in the diet, and when the cells are undergoing fatty degeneration for some reason. The two types studied here are the fat converted from sugar and the fat mobilized during starvation, since it was believed that these two methods would involve little, if any, pathological change in the cells.

It is a long-known fact that the liver stores a large proportion of sugar as fat when the diet contains an excessive amount of carbohydrate. The conversion is probably a method of conserving food and preventing hyperglycemia, since the ability of the liver to store glycogen is limited. The diet used was devised by Barrett, Best and Ridout ('38) and is low in choline and protein which are the most effective lipotropic factors, that is, substances which prevent the accumulation of fat in the liver. Consequently, much of the fat stays in the liver, at least for the first 2 weeks of feeding. The fat appearing in the liver during starvation is mobilized from the body depots in response to the body's energy needs. In my preparations, the presence of fat globules in the blood of the liver indicates such mobilization.

Fat droplets appear in the liver within a day or two after the mouse has been placed on the high-sugar diet. At first, they are distributed uniformly in the lobule or slightly centrally. With longer feeding, these droplets become large and often confluent in all cells except those immediately adjacent to the central vein of the lobule. Best and Campbell ('38) have noted that it is characteristic of fat globules deposited in the liver on a low-choline diet to become confluent. After 2 weeks of the diet, the fat is concentrated in the middle and peripheral zones of the lobule. On the other hand, the fat deposited in the liver cells during starvation occurs in the form of discrete droplets and thus, when crowded, gives the liver a honey-combed appearance in paraffin

sections. The fat may be distributed uniformly throughout the lobule, but it is frequently more concentrated in the central half of the lobule in contrast to the more peripheral concentration of fat in animals long on the diet.

The livers of animals more than 2 weeks on the diet appear to contain less fat than those killed earlier. This apparent reduction of the fat content may not be a real one, for Grafflin ('40) found that the amount of sudanophil fat is not necessarily a good index of the amount of fat which can be extracted biochemically. However, Hodge et al. ('41) found that the amount of neutral fat which could be extracted did correlate with the amount of fat appearing in histological preparations, although the amount of cholesterol and phospholipid did not. If the decrease in fat is real, there is a reduction either in the amount of sugar converted to fat or in the amount of fat remaining in the liver after conversion. It is suggested that this reduction may be an expression of an adaptation to the diet.

From a comparison of the sudan IV and Heidenhain preparations, there is no apparent relation of mitochondrial swelling and vesiculation to the deposition of fat. With the diet, the mitochondria in the cells of the peripheral and middle zones become shortened and often swollen and vesiculated, while those in the central-zone cells are filamentous throughout the experiment. The initial deposit of fat is quite uniform or is slightly central and yet the swelling of the mitochondria begins in the peripheral zone and proceeds centrally. Thus there would seem to be no active relationship between swelling of the mitochondria and the deposition of fat. Furthermore, the vesiculation of the mitochondria does not occur until after the deposition of fat in the males on the diet, although it does occur early in the females. After starvation, also, the mitochondria may be enspherulated throughout the lobule, but again the more marked reaction is in the peripheral zone and the fat tends to be concentrated in the central zone of the lobule. Consequently, neither the zone nor time relationships substantiate the claim of Kater and Smith ('32) that the

vesiculated mitochondria synthesize and deposit fat within themselves or the assertion of Noël and Pallot ('34) that the heavy, swollen mitochondria elaborate fat on their surfaces. It is perhaps worthy of mention that both of these hypotheses were based partially on the study of osmic acid preparations, and other observers feel that these techniques do not give a sufficiently precise picture to warrant such conclusions (Hoerr, '36; MacCardle, '37).

In the animals fed the diet for a week and in the starved animals, also, the mitochondria become enspherulated and often vesiculated, as stated above. Whether this reaction of the mitochondria is associated with a specific function of the cell or not is difficult to determine. It is possible, for example, that it is associated with the deposition or secretion of some substance, or it might merely be the response of the mitochondria to a change in the cytoplasm which is effected by many different causes. Many cytologists have suggested that the enspherulation of mitochondria is an indication of a reduced rate of cellular oxidation (Cowdry, '24), and thus the enspherulation following the diet and following starvation might in both instances be attributed to a lowered rate of metabolism of the liver cells. In the one case the enspherulation accompanies the conversion of sugar to fat and in the other the utilization of fat mobilized from the body depots. Since the two processes are essentially different, biochemically, the great similarity of the cytological picture would certainly tend to indicate that the mitochondria play no specific rôle in these processes.

After the animals have been on the diet 2 weeks, the mitochondria, even of the peripheral zone of the lobule, are no longer swollen and vesiculated but are fine, elongated filaments. In animals on a balanced diet the cells in the liver may be considered to be in a state of equilibrium. Hence, when such animals are killed in the non-storage phase the mitochondria are not swollen, even though they do become swollen and vesiculated in the outer part of the liver lobule in the normal physiological processes associated with the

absorption and utilization of food. However, when the animals have been on the high-sugar diet 1 week or are starved, there is an accentuated tendency for them to be swollen even though the animal is killed in the non-storage phase. This may be a result of a change in metabolism. The return of the mitochondria to their customary diameter in animals fed the diet for more than 2 weeks could be interpreted as an indication that the liver has become adjusted to the unbalanced diet.

The liver cells of animals more than 2 weeks on the diet contain large, irregular spaces other than those filled by fat globules. The mitochondria in such cells, although elongate, are not sharply differentiated from the cytoplasm and appear clumped. If, as suggested above, the liver cells are no longer converting sugar to fat, a hyperglycemia may exist, and the mitochondria would be clumped in response to this, since it was pointed out by Steffens ('41) that the liver cell mitochondria clump in a hyperglycemic animal. Furthermore, Emmel ('40) described coalesced mitochondria in cells of kidney tubules recovering from ischemia, and he suggested that such fusion is the result of a change in the water distribution of the cell associated with a reduction in pH. The spaces which appear to be watery in my preparations might be a further indication of such an upset. Possibly, then, both hyperglycemia and an altered water balance may be affecting the liver-cell structure when the animals have been on the high-sugar diet for several weeks, and this is reflected in the clumping of the mitochondria of the peripheral zone where the watery vacuoles occur.

According to my observations, there is some indication of a sex difference in liver-cell metabolism and structure. This may not be statistically significant in view of the small number of animals studied, but it does agree with the sex difference previously suggested by physiologists. The more persistent accumulation of fat and swelling of the mitochondria in the females and the greater amount of fat in the livers of females than males following the diet suggest a difference in carbo-

hydrate and fat metabolism. Deuel, Hallman and Murray ('37), McLean, Ridout and Best ('37), Best and Campbell ('38), and many others have found that the female has a greater tendency than the male to get a fatty liver under several experimental conditions and is more prone to fasting ketonuria. In fact, Deuel and coworkers have suggested that there is a qualitative as well as a quantitative difference in the fat metabolism of the two sexes, but naturally a study of cytological preparations could yield no information of this sort.

Several types of cells that are unlike typical liver cells are found in liver preparations from animals which have been on the diet a short while or have been starved. These are called "crimson" cells, "black" cells, and "clear" cells from their appearance in fixed preparations. "Crimson" cells, which stain darkly with sudan IV (see p. 174), are scattered throughout the lobule in many preparations. It was thought at first that these cells might correspond to the "black" cells in hematoxylin preparations, but further study showed that they did not have the same distribution. At present no suggestion can be made as to their significance. In hematoxylin preparations, the so-called "black" or dark cells stain intensely with the hematoxylin and appear as though the mitochondria had either dissolved in the cytoplasm or swollen and fused. Mayer, Rathery and Schaeffer ('14) call such cells homogenized. They correspond in appearance to moribund cells described by other investigators. Emmel ('40) describes the fusing of mitochondrial spherules in moribund cells in the distal tubule of the kidney following ligation of the renal artery; Duthie ('35) has described an intense staining of the cytoplasm which masks the presence of discrete mitochondria in dying cells in liver transplants. Furthermore, Clara ('32) considered similar "black" cells occurring in the dog liver following phosphorus poisoning to be dying since they take up trypan blue diffusely. It might be added that Richardson ('34) found that the "degeneration granules," so often described in dying tissue culture cells, stain

intensely with hematoxylin following fixation with Zenker-formol. It would be difficult to determine whether the "black" cells in my preparations contain crowded and coalesced mitochondria or "degeneration granules." Other unusual cells in the livers of animals on the diet for a short while and in the starved animals are the "clear" or light cells containing few or no apparent mitochondria. Tissue culture studies have revealed that the mitochondria in dying cells often fragment and disappear, a process known as chondriolysis (Richardson, '34). However, Clara ('34) found that the light cells in the liver do not take up trypan blue at all, which may indicate that they are not moribund in this organ. Scharrer ('38) thinks that both the light and the dark cells found in liver preparations are artefacts produced by cutting the organ before fixation. It is true that darkening of the cells on the surface of the block does occur in a few of my preparations, but the cells under consideration are isolated and in no constant relationship with the connective tissue which might transmit the stress. The picture Scharrer gives is of aggregates of cells on the surface of the block and in close juxtaposition to the connective tissue strands, while the cells here under consideration are isolated, which would be an indication that the reaction is a local one. Fischler and Roeckl ('38) likewise deny Scharrer's contention that all dark cells are fixation artefacts.

Whether or not the cells in my preparations, similar in appearance to moribund types, are truly abnormal cannot be ascertained. Such cellular changes might occur in healthy physiological processes, and their appearance in so-called normal animals might indicate an ordinary activity rather than cell death. Even if they do indicate abnormality, there is no proof that they are moribund, since only occasional signs of cellular debris and phagocytosis appear. These cells might rather be in a state of reversible necrobiosis, reflecting the strain placed upon the liver by the suddenly altered metabolism of the organism. In such a case, the weaker cells of the population may undergo initial necrobiotic changes but

then recover after a period of adaptation. Fischler and Roeckl ('38) offer the interesting suggestion that "black" cells indicate the strain on the organ and that they may proliferate in the event of liver restoration or may degenerate, depending on the subsequent conditions. If the cells die, they must be replaced by mitosis or by hypertrophy of the remaining cells, or else the liver must become smaller. What occurs cannot be determined; there are no mitotic figures in any of the preparations, and it does not seem feasible to make valid cell measurements for an indication of hypertrophy because of the variable amount of fat in the cells. For the same reason, weighing the liver would be of little significance. Whatever may be their significance, the "black," "clear" and "crimson" cells have either disappeared or been restored to a normal appearance after several days of the diet, and liver sections again have a uniform appearance.

SUMMARY AND CONCLUSIONS

1. The livers of mice on a high-sugar diet become very fatty within a week. The fatty condition is somewhat reduced after 2 weeks, and watery vacuoles appear in the cells at this time.

2. The superposition of a 48-hour fast upon the diet augments the fatty appearance of the liver.

3. The mitochondria of the liver cells, under both experimental conditions, become short, swollen and sometimes vesiculated. After more than 2 weeks of the diet, however, they become finer and elongated but are often coalesced.

4. No direct relationship between the mitochondria and the deposited fat is apparent. It is suggested that the enspherulation of the mitochondria may rather result from a change in cellular metabolism under the conditions of these experiments.

5. When the animals are restored to a balanced diet, the fat is lost from the liver and the mitochondrial picture is restored to approximately normal within a week.

6. There are indications of a sex difference in carbohydrate and fat metabolism, with the female mouse being more

susceptible to the high-carbohydrate diet and to starvation than the male.

7. The significance of "crimson" cells, "black" cells and "clear" cells is discussed.

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PLATES

EXPLANATION OF PLATES

Figures 1 through 9 are of mitochondrial preparations from liver tissue fixed in formalin, postchromed, cut at 3 micra and stained with Heidenhain's iron-alum hematoxylin. Figures 10 through 12 are from fat preparations fixed in formalin, cut on the freezing microtome at 10 micra, and stained in sudan IV and Delafield's hematoxylin. All photographs were taken using apochromat objectives and compensating oculars. Figures 1 and 11 were taken with a 16 mm. (10 ×) objective; all others were taken with the 2 mm. oil immersion objective (90 ×). The photographs of Heidenhain preparations were taken with no filter, those of the sudan preparations with a Wratten X_1 green filter.

PLATE 1

EXPLANATION OF FIGURES

1 Male, diet 1 day. Zonation of liver lobule in Heidenhain preparation, with a few rows of cells around the central vein stained less intensely. Magnification, × 90.

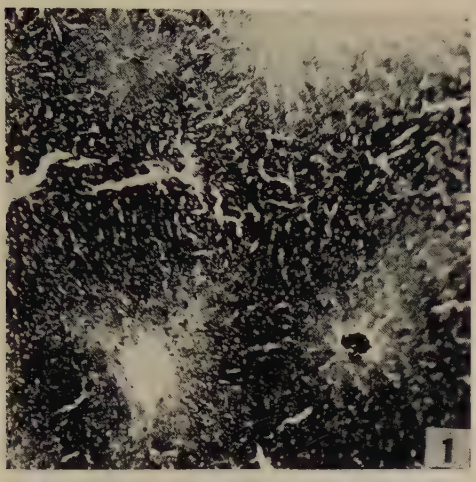
2 Female, diet 4 days. Middle zone of lobule, showing vacuolated cells containing beaded, filamentous mitochondria. Some appear swollen. The mitochondria are oriented perpendicular to the sinusoids. Magnification, × 1300.

3 Female, diet 4 days. Peripheral zone of the lobule, showing irregularly swollen mitochondria. Magnification, × 1300.

4 Male, diet 14 days. Peripheral zone of the lobule, showing filamentous and spherical mitochondria of a typical diameter for this zone. Magnification, × 1300.

5 Male, diet 4 weeks. Cells contain fine, filamentous mitochondria in the central half of the lobule shown in the upper part of the photograph; mitochondria in the peripheral half of the lobule, shown in the bottom part of the photograph, are sometimes discrete filaments but generally coalesced. Magnification, × 1300.

6 Female, starved 48 hours. Peripheral zone of the lobule, showing swollen spherical mitochondria. Two "black" cells with a homogeneous appearance. Magnification, × 1300.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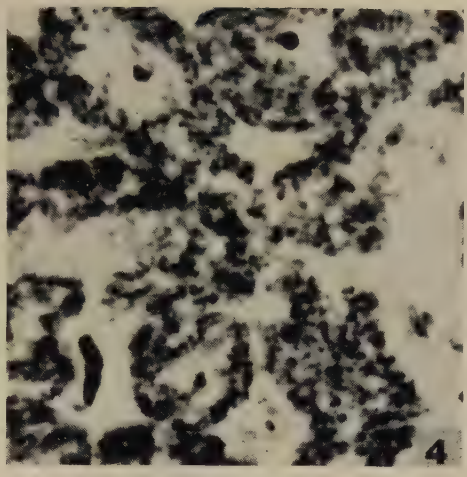
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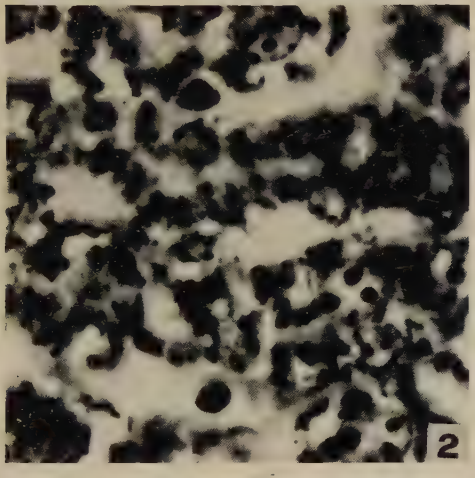
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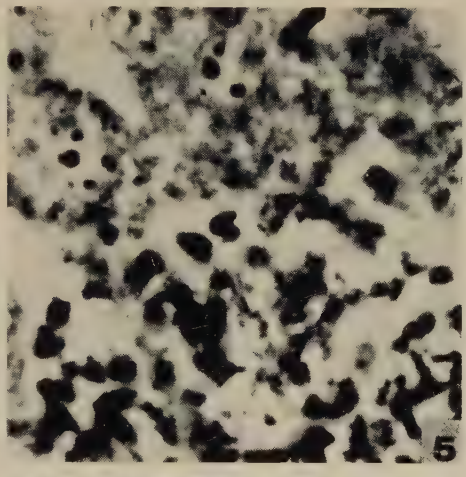
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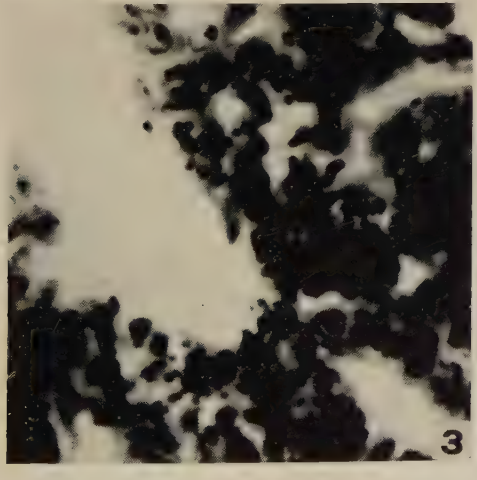
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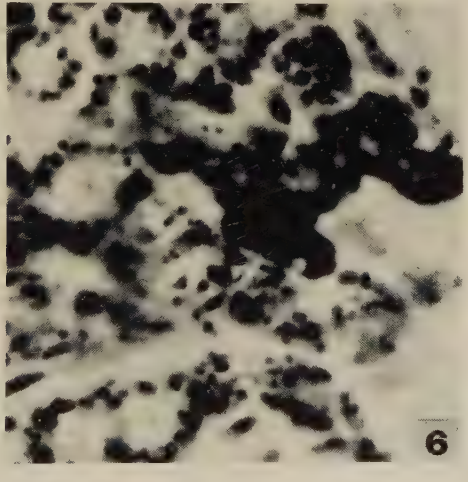
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PLATE 2

EXPLANATION OF FIGURES

7 Female, diet 3 weeks followed by a 48-hour starvation. Central zone of the lobule. Cells adjacent to the central vein contain only a very few mitochondria and are thus "clear." All cells of the lobule in this preparation are honeycombed. Magnification, × 1300.

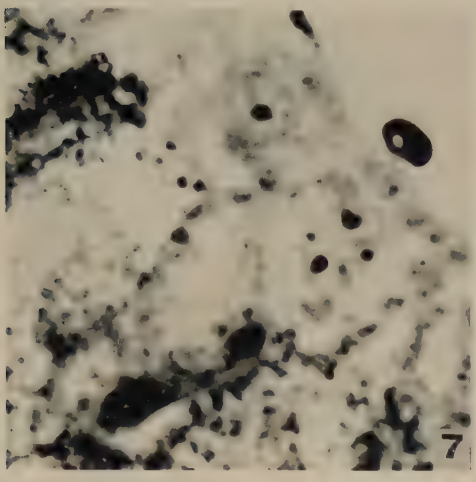
8 Male, after 2 weeks of the diet and 1 week of recovery. Central zone of the lobule. The mitochondria are fine, beaded filaments. Magnification, × 1300.

9 Male, after 2 weeks on the diet and 1 week of recovery. Peripheral zone of the lobule. The mitochondria are heavy, beaded filaments. In the lower left hand corner is a "black" cell containing large, fusing mitochondria; next to it is a "clear" cell containing only a few, pale spheres. Just above the "clear" cell is a cell containing enlarged mitochondria which is transitional to the "black" cell. Magnification, × 1300.

10 Male, diet 5 days. Sudan preparation. Peripheral zone of the lobule, showing fat droplets of various sizes filling all cells. One "crimson" cell. Magnification, × 567.

11 Female, starved 48 hours. The fat is particularly concentrated in the central half of the lobule. Magnification, × 90.

12 Male, diet 3 weeks followed by a 48-hour starvation. Central zone of the lobule. Large fat droplets; several cells contain watery vacuoles. Magnification, × 567.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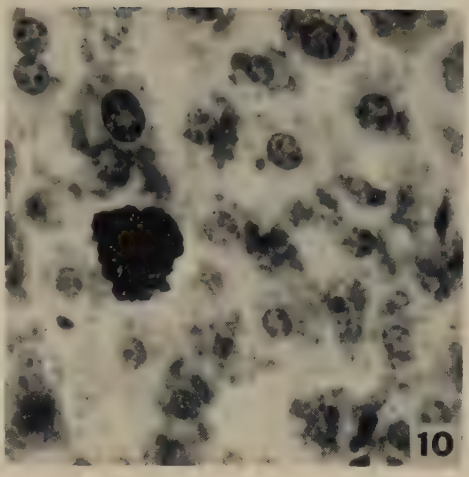
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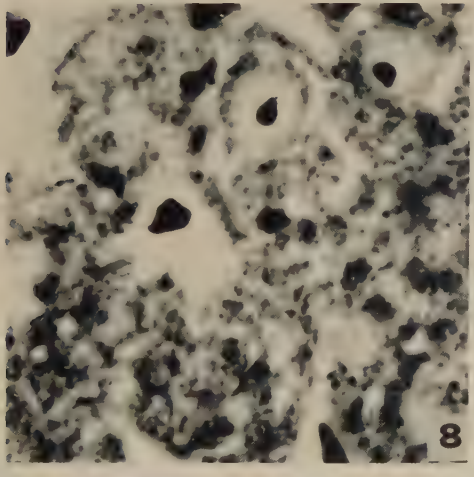
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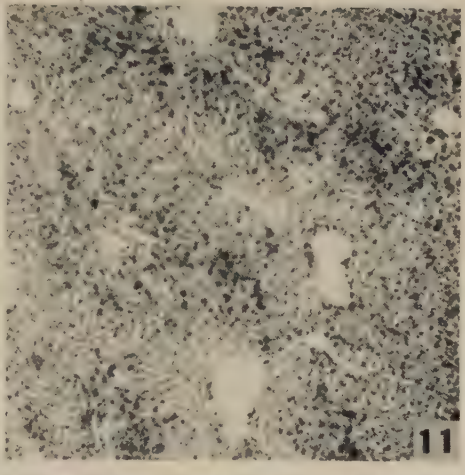
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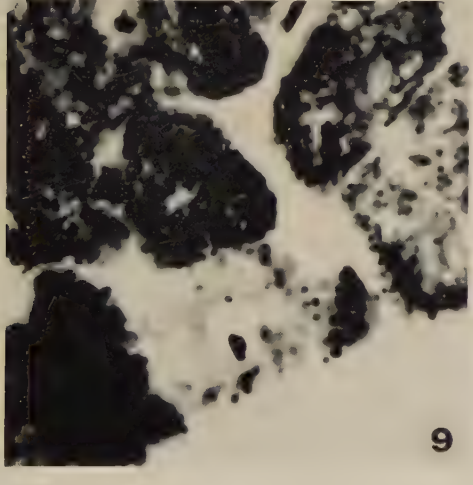
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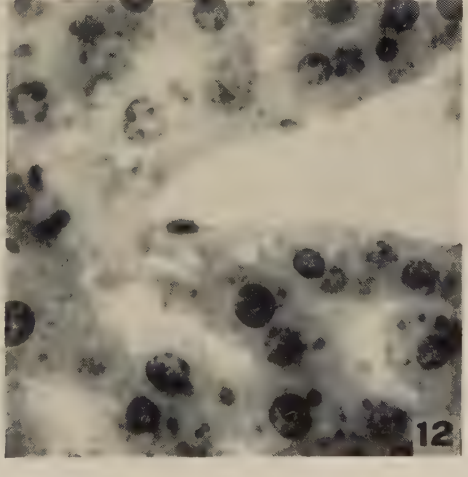
12

THE PHOTODYNAMIC ACTION OF NEUTRAL RED ON EMBRYONIC CHICK CELLS GROWN IN VITRO

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ONE TEXT FIGURE AND THREE PLATES (TWENTY FIGURES)

The study of the photodynamic reaction of cells under experimental conditions may help to elucidate the more general problems of photosensitization. Photosensitive substances not only exist normally in the tissues of the body but they may be introduced during the therapeutic treatment of disease. Although investigators (Blum, '41) do not yet agree upon the mechanism by which the photodynamic reaction becomes effective there is increasing evidence that the morphological responses of cells resemble closely those induced by various chemical (narcotics, carcinogenic substances) and physical (heat, radium, x-rays) agents. We can only postulate that the similarity in the visible responses of cells to such varied modifying agents depends in some way upon the specific organization of protoplasm.

The systematic investigations of Tennent ('42) show convincingly that dilute solutions of various dyes, non-toxic in darkness and in diffuse light, become highly toxic to echinoderm eggs (Lytechinus variegatus) when they are exposed to sunlight for very brief periods of time. Regarding the photodynamic reaction of neutral red he stated ('38) "that is would be possible to make a phase-by-phase comparison of the photodynamic effect of neutral red on cell division with the phases obtained by Politzer and others after x-ray irradiation." His students, Frankston ('39) working with amphibian cells and Patterson ('41) using barley root tips, have presented such phase-by-phase comparisons.

The present investigation, also suggested by the late Prof. D. H. Tennent, was undertaken in order to observe the immediate effects of the photodynamic reaction of the dye, neutral red, upon the morphology of cells from a warm blooded vertebrate. Tissue cultures of embryonic chick heart were chosen as the experimental material. Similar material was used by Strangeways and Oakley ('23–'24) who described the immediate changes in cells after exposure to soft x-rays. In 1933 Whitman reported upon the effects of radium irradiation on tissue cultures of normal and malignant cells. Lewis ('35) studied the photosensitivity of embryonic chick cells grown in a medium containing methylcholanthrene, one of the most active of the known carcinogenic substances. In many instances the cell abnormalities found in the experimental cultures now under consideration resemble strikingly those described by these investigators.

Grateful acknowledgment is made to Dr. William L. Doyle of Bryn Mawr College for his helpful criticism of the manuscript and to Mr. B. B. Varian of the Department of Anatomy, University of Pennsylvania for the photomicrographs.

MATERIAL AND METHODS

The technique of culturing involved no unusual features. The double cover glass method was employed and the cultures were inverted during the incubation period of 48 hours at 37½°C.

The culture medium consisted on one part of a 0.15% heparined plasma diluted in the ratio 2:1 with Tyrode solution and two parts of embryonic juice diluted 1:1 with Tyrode solution. For the experimental cultures a stock solution of 0.03% neutral red (Grübler's vital für Inject.) in Ringer's solution was prepared, autoclaved and stored in a refrigerator. At the time of culturing one part of the stock neutral red solution was added to nine parts of Tyrode solution. The embryonic juice for the experimental cultures was diluted with an equal volume of the neutral red — Tyrode solution. The

final dilution of approximately 1:100,000 neutral red was obtained when 2 drops of embryonic juice were combined with 1 drop of diluted plasma.

The Champy-Kull process was employed routinely as the method of fixation. In toto mounts were prepared and stained in acid fuchsin, toluidin blue and aurantia according to Lee ('37). The Feulgen technique, following fixation in a solution of 6% corrosive sublimate was applied satisfactorily to twelve cultures. A number of cultures were imbedded in paraffin for sectioning but the data from these are not yet available.

At the time of irradiation the cultures of 48 hours incubation were taken to the dark room. Following exposure to visible light they were returned immediately to the incubator. The source of non-filtered light was a 1000-watt General Electric light bulb (PS-52 Mazda C, clear) with white porcelain reflector. The intensity of the light was increased in successive experiments by decreasing the distance from the light bulb to the cultures. A General Electric exposure meter, with calibrated filters was used to determine the relative intensity of light at different distances.

For irradiation the cultures were placed on a heavy aluminum tray, the surface of which was covered with moistened paper. To keep the temperature below 39°C. ice cubes were placed upon the moistened paper but were not permitted to come in contact with the culture slides. Precautions were taken in recording the temperature. The bulb of a thermometer was incased in a stoppered tube which contained approximately the volume of air confined in the sealed cavity of a culture slide. In a single experiment the accuracy of the incased thermometer was checked against temperature determinations obtained from a thermocouple set up in a dummy slide. In the experiments reported here the temperature did not rise above 39°C. but varied between 30°C. and 36°C. for the greater part of the time during any given period of irradiation.

Table 1 summarizes the experimental procedure.

TABLE 1

Experimental data.

SERIES	DISTANCE	RELATIVE LIGHT INTENSITY [1]	TEMPERATURE VARIATION °C.	PERIOD OF IRRADIATION		TIME OF FIXATION (MIN. AFTER END OF IRRAD.)	EXPERIMENTAL CULTURES	CONTROLS		
				15 min.	20 min.			No irrad. No N.R.	Irrad. No N.R.	No irrad. + N.R.
1	35 cm.	Approximately 3000 ft. candles	25°–36.4°C.	12 cultures		45 min.	4			
						135 min.	4			
						195 min.	4			
					15 cultures	immediately	1	1	1	1
						45 min.	4			
						135 min.	5	2	1	2
						195 min.	3	1		
2	30 cm.	Approximately 4000 ft. candles	24.8°–36.9°C.	12 cultures		45 min.	3			
						135 min.	6			
						195 min.	3			
					15 cultures	immediately	1	1	1	1
						45 min.	3			1
						135 min.	6			
						195 min.	3		1	1
3	25 cm.	Approximately 5000 ft. candles	27°–35.7°C.	12 cultures		45 min.	4			
						135 min.	4			
						195 min.	4			
					15 cultures	immediately	1	1	1	1
						45 min.	4			
						135 min.	4			
						195 min.	4		1	1
4	20 cm.	Approximately 6000 ft. candles	23°–38.4°C.	12 cultures		45 min.	4			
						135 min.	4			
						195 min.	4			
					12 cultures	45 min.	4			
						135 min.	4			
						195 min.	4			

[1] A General Electric exposure meter with calibrated filters was used to determine the relative intensity of light at distances of 20–35 cm.

OBSERVATION OF LIVING CULTURES

In each experiment observation of living cultures started 15 min. following the return of irradiated cultures to the incubator and continued for 3 hours. During this period cultures were withdrawn for fixation at fixed intervals. The vital observations, therefore, form a composite picture from groups of cultures.

Individual experiments need not be discussed separately since the variations were quantitative rather than qualitative. The observable reactions of the cells were of the same nature in all the experimental cultures. Both the rate at which these reactions occurred and the number of cells affected in a given culture varied with the duration of exposure to light and in a smaller degree with the distance from the light. The effects of the photodynamic action were not wholly uniform throughout all parts of a single culture or in cultures receiving the same treatment. Two factors may account in part for this lack of uniformity: (1) the inherent variability of the biological material (the cultures were not homogeneous populations of cells) and (2) the possible uneven dispersion of light over the field.

The first observable reactions of the peripherally located fibroblasts were the retraction of cytoplasmic processes and the simultaneous appearance of blebs. The blebs varied in size, formed and disappeared rapidly. "Resting cells," as well as cells undergoing mitosis exhibited bleb formation (fig. 1). More severely injured fibroblasts became lobed. In some instances the contours of lobed cells smoothed out and rounded-up cells resulted. In contrast to these transformations easily followed under the microscope some cells exhibited a violent and excessive blebbing which resulted in sudden disintegration. For a time "rosettes" formed of cell fragments remained but these eventually underwent complete cytolysis. Fibroblasts and macrophages, to a greater degree, showed this reaction. The ultimate number of injured cells were present within 135 min. following irradiation.

There was a difference in the response of cells irradiated with an intensity of 5,000 foot candles or less (series 1, 2, and 3) and of those which received irradiation of greater intensity. In the former group formation of blebs following irradiation became evident at once whereas in the latter group a delayed reaction was apparent. Furthermore, a delayed response was not evident in cultures of series 3 which were irradiated for 15 min. but it was detected in cultures of the same series irradiated for 20 min. In the cultures of the latter group a temporary inhibition of the formation of blebs lasted for

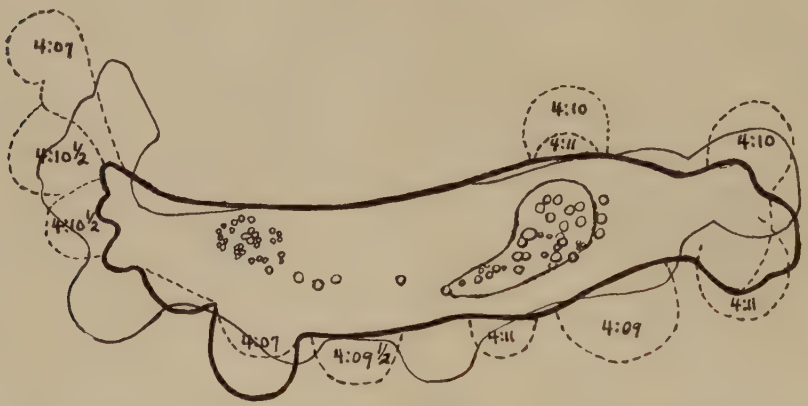

Fig. 1 Camera lucida drawing of a fibroblast from a living culture 106 min. following irradiation at a distance of 20 cm. for 20 min. Heavy contour line shows outline of cell at 4:06 P.M.; interrupted lines indicate appearance of cytoplasmic blebs; light contour line shows outline of cell at 4:30 P.M. Magnification approximately 1700 diameters. Reduction one fourth.

approximately 60 min. Following this period the least injured cells underwent typical blebbing, lobing or rounding-up. A considerable number of cells retained their typical shape. It seems probable that an irreversible gelation of the cytoplasm occurred in the latter case. Evidence from the fixed and stained cultures substantiates this view.

Heart muscle cells, fibroblasts and macrophages were the predominant types of cells in all 48-hour-old cultures. The heart muscle cells forming the central portion of the explant were surrounded by fibroblasts and migratory macrophages.

The contractile mechanism of the muscle cells was not damaged by irradiation of the intensities used in these experiments. Contractions of culture 17, series 4 were observed 115 min. after irradiation.

No consistent color changes in the neutral red vacuoles could be observed following irradiation. The vacuoles of the fibroblasts tended to lie scattered in the cytoplasm or to accumulate in the vicinity of the nuclei. Occasionally single cells were found in which blue-pink, red and yellow-red vacuoles existed simultaneously in the cytoplasm. Nuclear changes were difficult to observe in the living cultures. In some instances the chromosomes of irradiated dividing cells were more clearly visible than in non-irradiated cells.

CYTOLOGICAL EXAMINATION OF CONTROL CULTURES

Fixed material

The experimental procedure necessitated three sets of control cultures, namely: group 1, cultures which received no neutral red and were not irradiated; group 2, cultures which received no neutral red but were irradiated; group 3, cultures which received neutral red and were not irradiated (fig. 21).

The growth exhibited by all control cultures appeared normal for explants of 48 hours incubation. Fibroblasts formed a fairly uniform halo about the periphery of the explant. To form a basis for the study of the experimental cultures the control cultures were examined particularly for abnormalities exhibited by individual cells. Even under the most favorable conditions of life in vitro a certain number of cells undergo degeneration and consequently atypical cells may be found in any culture.

All phases of mitosis were encountered in all of the control cultures. The total number of mitoses from five cultures of each group was ascertained. Groups 1 (no N. R., non-irrad.) and 3 (N. R., no irrad.) each had approximately 500 cells undergoing division whereas over 650 mitoses were present in group 2 (no N. R., irrad.). It should be noted that the cultures comprising the latter group were fixed either im-

mediately or 195 min. following irradiation. Since there was no evident increase in the number of prophases in this group, it seems probable that irradiation alone, under the conditions described here, retarded the normal process of mitosis.

The multipolar mitoses (tetrasters) encountered in a single culture of group 2 have already been described (Stilwell, '40).

CYTOLOGICAL EXAMINATION OF EXPERIMENTAL CULTURES

Fixed material

Cultures irradiated at distance of 35 cm. for 15 min. (lowest intensity). Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. The results of irradiation are found in nuclear as well as cytoplasmic variations from normal conditions. Only a mild degree of cytolysis took place and consisted for the most part, of the appearance of small cytoplasmic blebs. Only occasional lobed fibroblasts and rosettes (fig. 2) are present. No prophases and only atypical metaphases are to be found. In the latter (fig. 15) the spindle is absent; the chromosomes are clumped (pycnotic). Injury to the spindle may possibly be temporary in nature since two metaphases with slender spindles are present in a culture fixed at 195 min. following irradiation. Anaphases and early telophases are lacking. Daughter cells, connected by a cytoplasmic strand with midbody are more numerous in the 45-min. fixation group than in the later groups. An occasional binucleated daughter cell connected with a non-nucleated portion (fig. 3) may be found. Binucleated cells may also arise through the failure of the cytosome to divide (fig. 4). Figure 5 shows a lobed nucleus with a detached fragment connected by a delicate thread to the nuclear membrane. A detail (fig. 16) from culture 29 which was fixed 195 min. after irradiation shows rounded-up fibroblasts and a partially disintegrated fibroblast.

Cultures irradiated at distance of 35 cm. for 20 min. Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. The reaction was greater than in

the preceding group. Not only are more cells affected but the degree of injury is greater, i.e. lobed fibroblasts are more numerous than those with cytoplasmic blebs. The remains of a large number of macrophages at the inner edge of the marginal growth are present as clusters of cell fragments (rosettes). Qualitatively the results of irradiation are of the same nature as those in the preceding group.

Cultures irradiated at distance of 30 cm. for 15 min. Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. No additional abnormalities were found. Metaphases with clumped chromosomes are of frequent occurrence. Daughter cells are more plentiful in cultures fixed at 45 min. than in those killed at a later time. With the exception of a single cell in late telophase, atypical metaphases and daughter cells are the only stages of mitosis present. Interphase nuclei exhibiting chromatin bodies on the inner surface of the nuclear membrane (figs. 6 and 7) which are rare in cultures irradiated at 35 cm. are more numerous in this group of cultures.

Cultures irradiated at distance of 30 cm. for 20 min. Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. Various degrees of cytolysis are exhibited by a larger number of cells than in cultures of the previous groups. No normal mitoses are present. A single fibroblast (fig. 8) exhibits a bridge of chromatin between the astral masses of chromatin. The nuclei of a few daughter cells appear to contain vacuoles (fig. 9). At 195 min. peripheral fibroblasts which retain their characteristic shape appear enlarged with vacuolated cytoplasm. Figure 10 illustrates an abnormality encountered rather frequently in this group of cultures as well as in other groups. The daughter cells appear to be re-uniting; a thread with midbody lies outside of the main cytoplasmic band connecting the cells.

Cultures irradiated at distance of 25 cm. for 15 min. Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. The reaction in this group is more marked than in the previous groups. Few, if any, cells

of the marginal growth are unaffected (fig. 17). Lobed and rounded-up cells predominate. The surface of fibroblasts retaining their cytoplasmic processes is roughened. Small cytoplasmic blebs cover the surface of daughter cells. The cytoplasm of fibroblasts of more or less normal shape is filled with coarse, angular granules. The nuclei of these cells are condensed and stain poorly. In cultures fixed 195 min. after irradiation cells with ruptured nuclei appear. In such cells (fig. 11) discreet masses of nucleoplasm lie just beneath the plasma membrane. Distorted metaphases (fig. 12) with clumped chromosomes are present.

Cultures irradiated at distance of 25 cm. for 20 min. Cultures in this group were fixed 45 min., 135 min., and 195 min. following irradiation. The reaction of this group is of particular interest. Typical cytolysis (blebbing and lobing) was temporarily delayed. In a single culture fixed immediately following irradiation all phases of mitosis are recognizable. With low magnification (16 mm. objective) cultures fixed at 45 min. appear more nearly normal than those which received less intense irradiation. In these cultures fibroblasts are for the most part typical in shape. Incompletely rounded-up metaphases are present (fig. 13). The condensed nuclei of resting cells stain poorly and no nucleoli are visible. In cultures fixed at 135 (fig. 18) and 195 min. the typical reaction (various degrees of cytolysis) is evident. Few, if any, cells are uninjured.

Cultures irradiated at distance of 20 cm. for 15 min. Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. In these cultures the majority of cells retain their typical shape although their surfaces are roughened by small blebs. In cultures fixed 45 min. after irradiation the metaphase chromosomes are less pycnotic than they are in cultures which received less intense irradiation. Figure 14 shows a fibroblast with astral groups of chromosomes connected by chromatin bridges. The destructive effects of irradiation become pronounced in cultures fixed 135 min. and later. In these the nuclei of resting cells are enlarged

and stain poorly; vacuolization of the cytoplasm is marked. At 195 min. after irradiation the cells either exhibit increased vacuolization of their cytoplasm or undergo blister cytolysis.

Cultures irradiated at distance of 20 cm. for 20 min. (most intense irradiation). Cultures from this group were fixed 45 min., 135 min., and 195 min. following irradiation. In cultures fixed 45 min. after irradiation metaphases, telophases and daughter cells are present (fig. 19). The nuclei of resting cells in these cultures have lost their capacity to stain. At 195 min. after irradiation the cells either show increased vacuolization of their cytoplasm (fig. 20) or undergo blister cytolysis. The presence of cellular debris in cultures fixed at this time is further evidence of the degeneration and disintegration of cells.

DISCUSSION

Three experimental cultures (culture 31 of series 1, 2, and 3) were fixed immediately following a 20-min. period of irradiation. All phases of mitosis could be found in these cultures. In cultures fixed at a later time (45 min., 135 min., and 195 min.) only abnormal metaphases, rare telophases and numerous daughter cells (connected by a cytoplasmic thread) were present. It thus becomes evident that under the conditions of the experiments reported here, the progress of cell division is interrupted at two stages by the photodynamic action of the vital dye, neutral red. Cells were prevented from entering mitosis and cells in metaphase were injured to a degree precluding recovery. It is difficult to detect injuries of cells in which the prophase is about to be initiated. Figures 6 and 7 show cells with peculiar chromatin particles adherent to the inner surface of the nuclear membrane. It is possible that such cells were about to enter mitosis at the time of irradiation. Cells remarkably similar to these are presented by Strangeways and Oakley ('23–'24) who call them "breaking down cells." They state that "these changes appear to take place in growing cells which have been damaged by exposure to x-rays and to develop when the cell is about to divide, . . ."

Nuclear fragmentation (fig. 5) also appears to take place in cells about to undergo prophasic changes. Nuclear fragmentation was found by Tennent ('42) in inseminated echinoderm eggs following the photodynamic action of the dye auramin O. In his figures 16 and 17 astral radiations from an intact nuclear membrane may be considered as indicative of the initiation of the prophase.

Since no prophases are present in cultures fixed 45 min. or later after irradiation cells in prophase at the time of irradiation must have progressed to metaphase. Abnormal and dying metaphases are numerous in all experimental cultures. In these cells pycnotic chromosomes form a deeply staining mass which may or may not retain its equatorial position (figs. 12, 13, and 15). No spindle substance is visible and in some instances cytoplasmic granules crowd upon the metaphasic plate of pycnotic chromosomes (fig. 18). It seems probable that the photodynamic action of neutral red produces irreversible alterations in the viscosity changes which normally take place during the progress of mitosis (Chambers, '17); Heilbrunn, '20, and others). A similar destruction of the spindle substance was found by Lewis ('35) in cells of chick embryos following the photodynamic action of carcinogenic substances (1:2:5:6 dibenzanthracene; 1:2 benzpyrene and methylcholanthrene).

With the exception of cultures of series 3 (20 min. irradiation) and series 4 typical anaphases and early telophases are rarely present in the experimental cultures. Examples of chromatin bridges between astral groups of chromosomes (figs. 8 and 14) have been found. Late anaphases with one or more deviated chromosomes are present but are extremely rare.

The frequent occurrence of daughter cells connected by cytoplasmic threads indicates that anaphases and telophases for the most part are not checked in their progress. This finding merely substantiates evidence obtained by other investigators who have found that these stages of mitosis are less susceptible to injury than the earlier stages (early prophase and metaphase) (Politzer, '25, '34; Ludford, '36;

Beams and Evans, '40; and others). Multinucleated cells occur not infrequently. Retraction of the daughter nuclei into a single cell (fig. 3) may possibly result from the influence exerted by chromatin bridges in the metaphase. In such cases the division of the cytosome is not necessarily inhibited. Binucleated cells may also arise through the failure of the cytosome to divide (fig. 4). Rosenfeld ('32) studying the action of ether on cells in mitosis attributed the failure of cytoplasmic constriction to appear to the extensive blebbing in the equatorial region of the cell and also to liquefaction of the whole cell body. Both of these factors may be operative following the photodynamic action of neutral red. The reuniting of daughter cells is suggested by such cells as figure 10 shows. The figure is also provocative when considered in relation to the role played by the "plasmagel band" in cell division (Lewis, '39). The appearance of vacuoles within degenerating nuclei of incompletely separated daughter cells is interesting (fig. 9). Pauli and Politzer ('29) describe "Kernvakuolization" of resting nuclei of epithelial cells of the cornea as a response following irradiation by cathode rays.

The sequelae resulting from the photodynamic action of neutral red are reflected in cytoplasmic as well as nuclear abnormalities. The non-specificity of the cytoplasmic reactions is noteworthy. Blister cytolysis, i.e. blebbing, lobing, etc. not only follows the photodynamic action of a number of dyes (Menke, '35; Tennent, '42) but is also a visible response of fibroblasts grown in vitro to the action of sublethal doses of ether vapor (Cash, '19; Rosenfeld, '32). The phenomenon is evidence of degenerative changes which may lead to the slow death of cells. Cash ('19) noted that active bleb formation was inhibited when rapid death followed upon exposure of cells to ether vapor. In the present experiments exposure of cultures to light intensities of approximately 5,000 foot candles for 20 min. or of approximately 6,000 foot candles for 15 min. or longer inhibited bleb formation (compare figs. 17 and 18). Cell death ensues in both cases. "Fixation" through the action of light has been reported by Blum ('30). In his experiments red blood cells in high dye concentrations at low

pH became refractory to hemolysis by low osmotic pressure when exposed to light.

Although the abnormalities produced in cells following the photodynamic action of neutral red closely resemble those induced by quite unrelated physical and chemical agents, it does not follow that the same cell constituent or fundamental metabolic process is destroyed by the different agents. It may be postulated that following the photodynamic action of neutral red a denaturation of cell constituents, presumably the cell proteins, occurs either slowly with concomitant visible protoplasmic reactions or with a rapidity which precludes visible responses of the cells. In both instances cell death is the end result.

SUMMARY

A study of the immediate responses of embryonic chick cells cultivated in vitro to the photodynamic action of neutral red has been made. The concentration of the dye (1:100,000) was the same in all experiments; the variables were light intensity and duration of exposure of the cultures. Observation of living cultures was continued for a period of 3 hours following irradiation. At stated intervals cultures were fixed for cytological study.

Individual experiments yielded quantitative rather than qualitative variations in results. The observable reactions of the cells involved both nuclear and cytoplasmic alterations. These reactions are non-specific in character resembling those produced by other agents capable of inducing cell abnormalities. The normal progress of mitosis was interrupted. Cells did not enter prophase. Cells in metaphase were injured to a degree precluding recovery. Division abnormalities included nuclear fragmentation, "breaking down cells," chromatin bridges in anaphases (rare), and formation of binucleated cells. The visible cytoplasmic response consisted of various degrees of blister cytolysis following exposure of cells to the lower range of light intensities (3,000–5,000 foot candles). Exposure to higher intensities (5,000–6,000 foot candles) caused a rapid denaturation of the cell constituents, presumably the cell proteins. In both instances cell death ensued.

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PLATE 1

EXPLANATION OF FIGURES

Drawings were made with the aid of an Abbe camera lucida and an achromatic oil immersion objective (Bausch and Lomb Co., 2 mm., N.A. 1.3) and a 12 × compensating ocular. Enlargement of 1900 diameters; reduction one-half.

2 From culture 25, series 1 (distance 35 cm.; 15 min. irrad.; fixed 135 min. following irradiation. "Rosette" of cell fragments resulting from sudden disintegration of cell.

3 From same culture as figure 2. Binucleated daughter cell attached to non-nucleated cytosome.

4 From culture 25, series 1 (distance 35 cm.; 15 min. irrad.; fixed 135 min. following irradiation). Binucleated cell resulting from failure of cytosome to divide.

5 From culture 29, series 1 (distance 35 cm.; 15 min. irrad.; fixed 195 min. following irradiation). Fibroblasts with nucleus in process of fragmentation.

6 and 7 From cultures 25 and 17 respectively, series 2 (distance 30 cm.; 15 and 20 min. irrad. respectively; fixed 135 min. following irradiation). Degenerating fibroblasts with chromatin particles on inner surface of nuclear membrane.

8 From culture 13, series 2 (distance 30 cm.; 20 min. irrad.; 45 min. following irradiation). Cell with telophasic masses of chromatin connected by chromatin bridge.

9 From culture 15, series 2 (distance 30 cm.; 20 min. irrad.; fixed 45 min. following irradiation). Degenerating daughter cells with nuclei containing vacuoles.

10 From culture 17, series 2 (distance 30 cm.; 20 min. irrad.; fixed 135 min. following irradiation). Thread with midbody outside of cytoplasmic band connecting daughter cells. Re-uniting of daughter cells?

11 From culture 28, series 3 (distance 25 cm.; 15 min. irrad.; fixed 195 min. following irradiation). Degenerating fibroblasts with masses of nucleoplasm against the cell membrane. A nucleolus appears as a solid body within one mass of nucleoplasm.

12 From culture 9, series 3 (distance 25 cm.; 15 min. irrad.; fixed 45 min. following irradiation). Degenerating fibroblasts showing mass of clumped chromosomes projected into cytoplasmic bleb.

13 From culture 13, series 3 (distance 25 cm.; 20 min. irrad.; fixed 45 min. following irradiation). Fibroblast in metaphase with mass of chromosomes without trace of spindle. Note typical cytoplasmic processes.

14 From culture 7, series 4 (distance 20 cm.; 15 min. irrad.; fixed 45 min. following irradiation). Early telophase with chromatin bridges connecting astral groups of chromosomes.

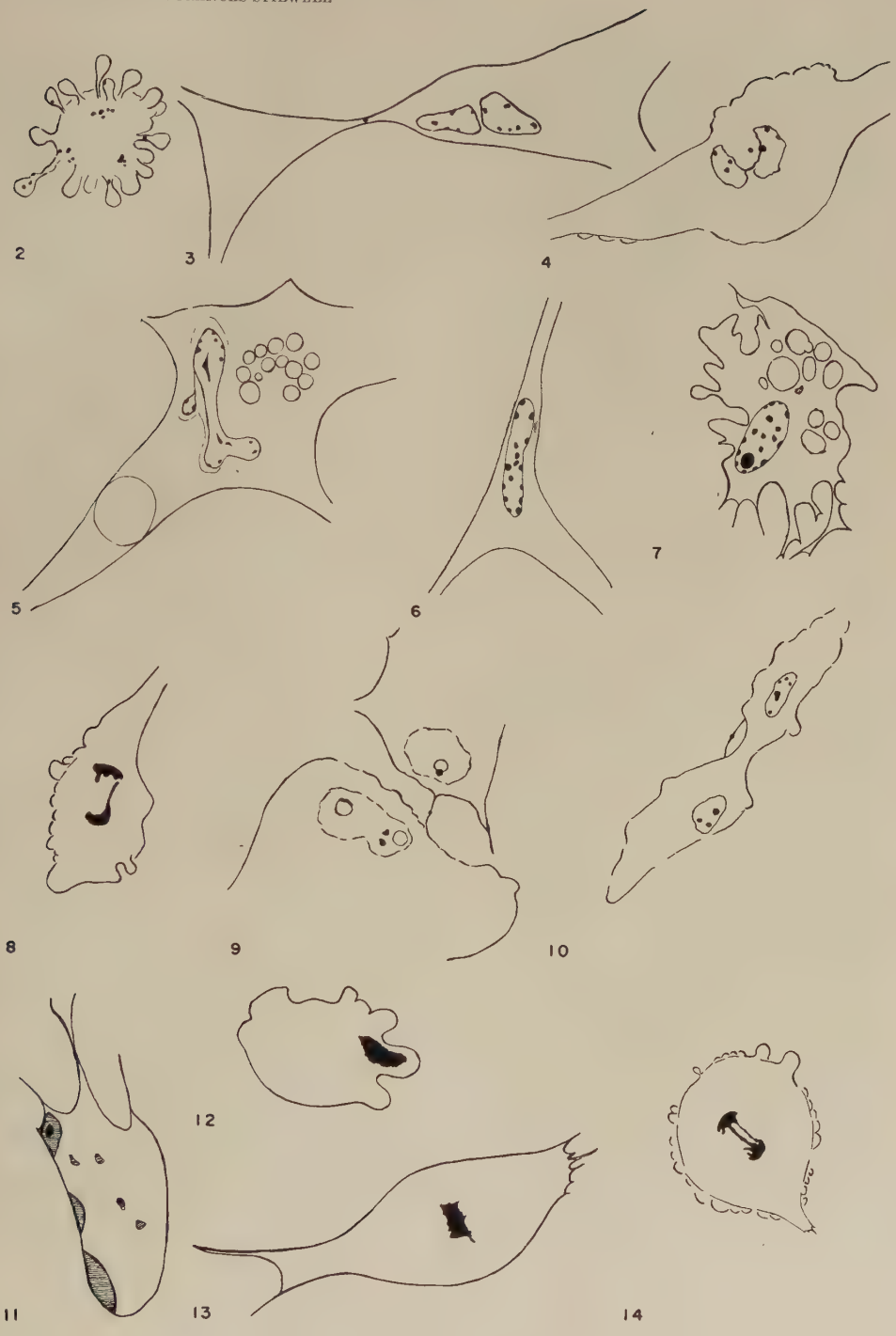
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PLATE 2

EXPLANATION OF FIGURES

All photomicrographs magnified 680 ×. Reduction one-third.

15 Detail from photomicrograph of culture 9, series 1 (distance 35 cm.; 15 min. irrad.; fixed 45 min. following irradiation). Rounded-up, blebbed fibroblast with pycnotic chromosomes lying in eccentric position. Note absence of spindle substance.

16 Detail from photomicrograph of culture 29, series 1 (distance 35 cm.; 15 min. irrad.; fixed 195 min. following irradiation). Number of rounded-up cells is greater than the number present in culture 9, series 1.

17 Detail from photomicrograph of culture 11, series 3 (distance 25 cm.; 15 min. irrad.; fixed 135 min. following irradiation). Fibroblasts are widely separated; destructive effect of photodynamic action of neutral red is evident.

18 Detail from photomicrograph of culture 17, series 3 (distance 25 cm.; 20 min. irrad.; fixed 135 min. following irradiation). Destructive effect is less evident than in culture 11, series 3. Rapid denaturation of protoplasm causes cells to remain typical in shape. Note fibroblast in metaphase.

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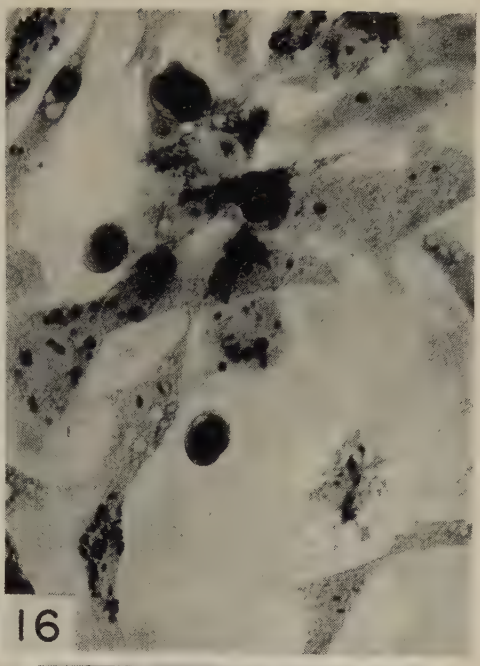
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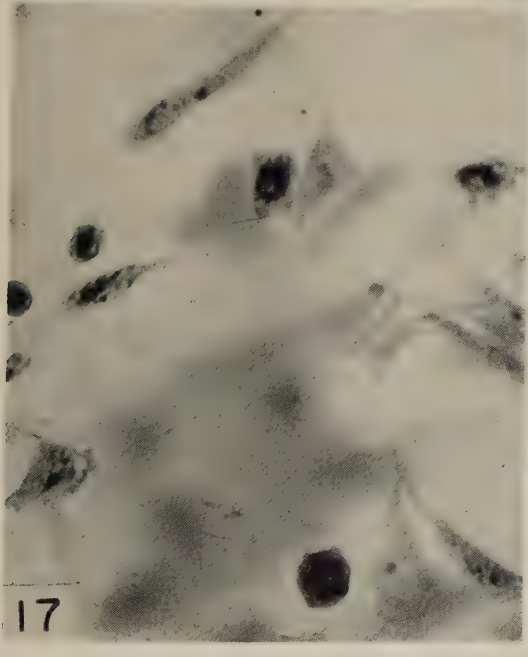
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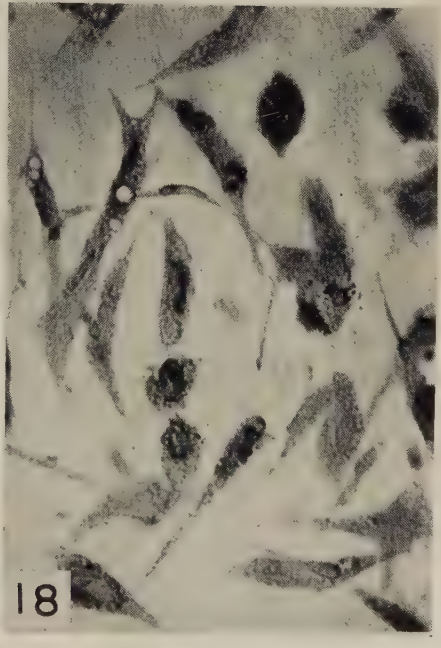
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PLATE 3

EXPLANATION OF FIGURES

19 Detail from photomicrograph of culture 15, series 4 (distance 20 cm.; 20 min. irrad.; fixed 45 min. following irradiation). Fibroblasts are typical in shape. Note metaphase, telophase and daughter cells. Nuclei of resting cells lack capacity to stain.

20 Detail from photomicrograph of culture 21, series 4 (distance 20 cm.; 20 min. irrad.; fixed 195 min. following irradiation). Dying fibroblasts of typical shape show degenerative changes consisting of vacuolization of cytoplasm and lack of capacity of nuclei to stain.

21 Detail from photomicrograph of culture 4, series 3 (non-irradiated; neutral red 1:100,000; 48 hrs. in vitro when fixed). Typical fibroblastic growth. Note mitotic figures (metaphase and late telophase).

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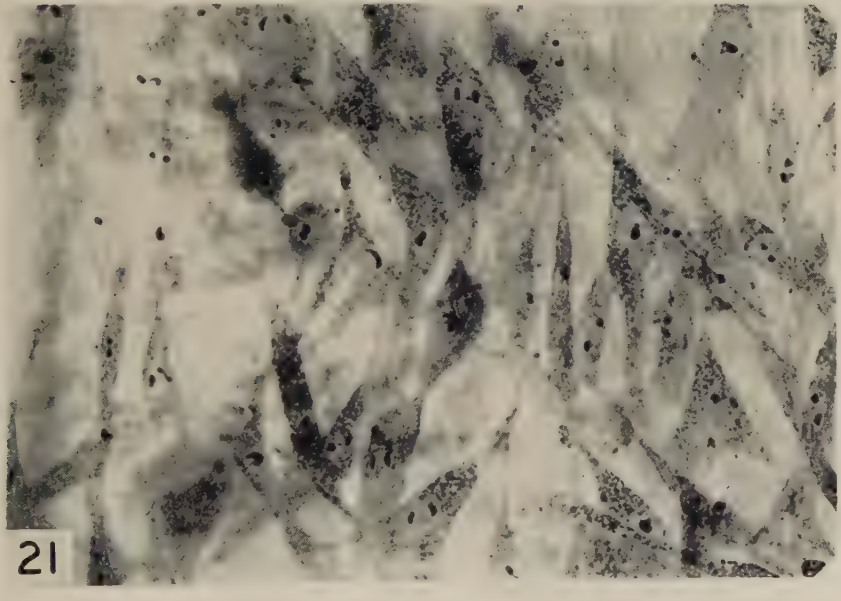
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A CASE OF EXTRAUTERINE PREGNANCY IN THE RABBIT

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ONE FIGURE

Ectopic gestation in the lower animals is rare. As early as 1898 Mandl and Schmit reported extrauterine pregnancies in such animals as the pig, dog, cow and horse, a great majority of the observed cases being secondary abdominal pregnancies. These authors suggested the possibility of true tubal pregnancies in rabbits. They believed that it is very easy to rupture the uterus in animals and extrude the fetus into the abdominal cavity.

The occurrence of a tubal pregnancy in a monkey was conclusively proved at autopsy of an animal who died suddenly in the zoo, and was immediately examined by Waldeyer in 1893.

Bell describes secondary abdominal pregnancy as quite common, especially in rabbits, although primary ectopic pregnancies are almost unknown. Occurrences of the former are due to the rupture of the uterine cornua with gradual extrusion of fetuses, the placenta in some instances remaining attached to the original sites, while in other cases the ova have formed fresh attachments to the peritoneum (usually in the neighborhood of the stomach, owing to the tendency the ovum would have to seek the lowest level). Bell considered that the only genuine cases of ectopic gestation in animals reported previous to 1910 were secondary abdominal implantations subsequent to rupture of the uterus. However, he concluded from careful study of his experimental animal that primary abdominal implantation can occur in lower animals.

Kamann, on the contrary, denied this. He described a specimen with one single fetus in the abdominal cavity of the rabbit which was proved to be a case of secondary implantation, since sections through the uterus showed a well-marked point of rupture.

Leo Loeb in his early experiments in 1908 had shown that it was apparently impossible to produce an extrauterine pregnancy experimentally in the guinea pig. Neither ligation of the fallopian tubes at the juncture with the uterus nor incision into the tubes or wall of the uterus at various periods of the sexual cycle led to the development of extrauterine pregnancy, although Loeb did produce decidual tumors by this procedure. Later, in 1915, he was able to produce experimentally the first stages of an extrauterine pregnancy in the guinea pig, even though the development of the embryo and placenta outside the uterus ended prematurely.

In this laboratory some 1500 mature, supposedly virgin, female rabbits have been examined for the routine Friedman modification of the Ascheim-Zondek pregnancy test. It was the policy to isolate these animals before use for a period of 3 weeks. Nevertheless, in perhaps 1% of them pregnancy was found, and in 100 cases no animal had other than a normal pregnancy.

When the reported animal was prepared for laparotomy under sterile conditions for Friedman ovulation test, following the injection of 5-10 cc. of urine 18 hours before, a hard mass was noted on the left side. This mass was freely movable and felt like an impacted loop of the large intestine. When the rabbit was opened for examination of the ovaries a light gray mass, consisting of two fetuses, was exposed. It was connected by a fine attachment to the mesentery along the small intestine as shown in (fig. 1). There was another attachment leading from between the two fetuses to the omentum 4 cm. from the spleen. These two fetuses have been turned completely over for purposes of illustration in order to show the small amount of attachment for the blood supply. The sac has also been cut away exposing the heads of two fetuses.

When the sac was completely dissected away, two small fetuses were found with their respective placentas. One of these was approximately a 29-day-old fetus with normal hair growth for this stage, while the other was about half normal

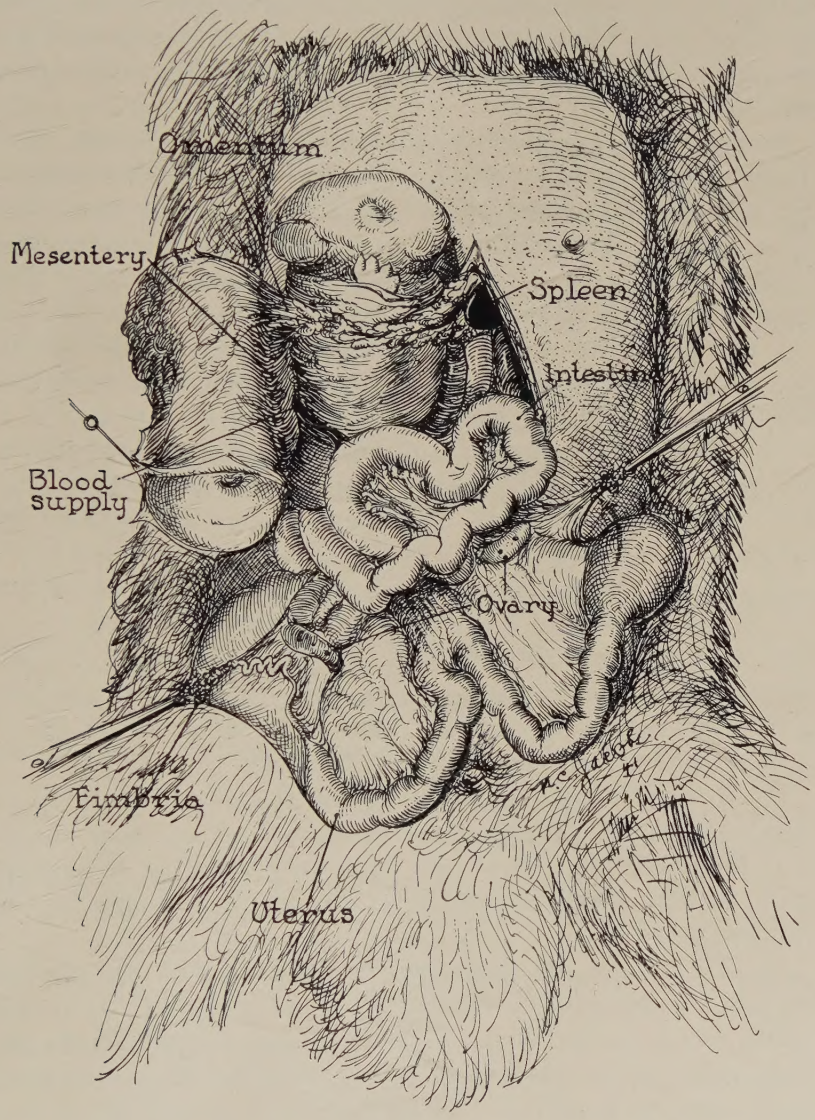

Figure 1

size. Both of these fetuses were somewhat dried, owing perhaps to the loss of amniotic fluid, but degeneration had not progressed to a marked degree. No gross or microscopic signs of tubal abortion or rupture of the uterus could be found. There was an enlargement of the left horn of the uterus near the distal end, which was identified as a spontaneous adenocarcinoma without metastases. (Willard Smith, data to be published.)

Sections were made of the uterus, tubes and ovaries. One of these taken of the uterus near the cervix shows typical four plus endometrial proliferation. The lining of the uterus in the other sections shows dilated glands lined with columnar epithelium. Several sections have a few secretory glands just beneath the layer of lining cuboidal epithelium.

A section of the ovary shows some round cell infiltration. The cells making up the stroma show beginning degeneration. Another section of the ovary contains corpus luteum tissue.

This is the first specimen of an advanced stage of extrauterine pregnancy in the rabbit which had been seen in this laboratory. Records and drawings in the department of anatomy revealed a similar case in a cat which had been opened for a physiologic experiment. In this case one fetus was found inclosed in a sac attached to the omentum. "Sections through the omentum and its attachment showed only fibrous tissue and no signs of a placenta. There was pus-like fluid inside the sac and the fetus showed signs of life." (Corner and Matthews, unpublished data.)

Dr. George Heckel of this department has noted a similar case in the rabbit, with the masses free in the abdominal cavity. It is his belief that these are the result of tubal or uterine rupture, with no actual implantation of the placenta in the abdominal cavity.

Shortly after the findings were reported, one of our medical students brought to the laboratory three fetuses from a wild rabbit which he had shot. These had been found free in the abdominal cavity, with no apparent attachment. All had become slightly mumified. One of these was 3×6 cm. in size and the other two were 2×4 cm.

Loeb ('13-'14) states that "In the human organism where extrauterine pregnancy occurs, decidua can to some extent form also at certain places outside the uterine mucosa." He therefore believes it is probable that one of the factors upon which the development of an extrauterine pregnancy depends is the capability of the tissue into which the ovum penetrates, to develop decidual tissue which presents to the ovum an adequate soil for development.

In extrauterine pregnancy the fertilized ovum may be arrested at some point between the ovary and the uterus and there undergo more or less complete development. In our animal there was enough decidual response on the part of the host to make possible the later stages of extrauterine embryonal development, even though the fetuses were completely outside the uterus.

A study of the slides made from the left horn of the uterus of our animal, as mentioned previously, showed the presence of a spontaneous tumor. From this one might assume that the escape of the ova did take place soon after fertilization.

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